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WASTEWATER USE IN THE PRODUCTION OF FOOD AND FIBER - PROCEEDINGS

Robert S. Kerr Environmental Research Laboratory

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16. Abstract

An interdisciplinary group of about 200 persons met to review the present base of scientific knowledge relating to benefits and constraints of using wastewaters for production of food and fiber. There were 27 papers presented by representatives from the fields of public health, engineering, agriculture, aquaculture, and other related scientific disciplines. Papers in two sections on potential restraints cover topics such as historical instances of disease transmission, possible transport of microbial pathogens through the food chain, legal implications, and sociological reactions. The aquaculture section deals primarily with experimental studies including such diverse approaches as culture of daphnia, salmon smolts, and water hyacinth. The agriculture section emphasizes the use of wastewater for crop production and the papers presented include case histories for long-term operating systems, as well as data from experimental studies. In addition to those papers presented at the conference, nine others have been included to make a total of 36 papers in the conference proceedings.

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WASTEWATER USE IN THE PRODUCTION OF FOOD AND FIBER--PROCEEDINGS

Proceedings of the Conference held at
Oklahoma City, Oklahoma
March 5-7, 1974

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ABSTRACT

A conference was convened to bring together an interdisciplinary group of investigators for the purpose of critically reviewing the present base of scientific knowledge relating to benefits and constraints of using wastewaters for production of food and fiber. Those gathered represented the fields of public health, engineering, agriculture, aquaculture, and other related scientific areas. There were 27 papers presented at the conference covering technical restraints, aquacultural approaches, agricultural approaches, nontechnical constraints, and new or integrated experimental systems. In addition to those papers presented at the conference, nine others have been included to make a total of 36 papers in this conference Proceedings.

Papers in the two sections on potential restraints cover topics such as historical instances of disease transmission, possible transport of microbial pathogens through the food chain, legal implications, and sociological reactions. The aquaculture section deals primarily with experimental studies including such diverse approaches as culture of daphnia, salmon smolts, and water hyacinth. The agriculture section emphasizes the use of wastewater for crop production and the papers presented include case histories for long-term operating systems, as well as data from experimental studies.

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FOREWORD

The topic of using domestic wastewater in the production of food and fiber is of great importance to three distinctly different groups in our society:

(1) Those persons living in arid or semiarid regions where economic growth, industry and agriculture hang on the thin thread of dwindling ground water supply. Reclamation and reuse of wastewater, even if only for irrigation of agriculture crops, would not only free a large percentage of treated water for municipal needs but would also minimize contamination of our primary water sources and provide cost benefits compared to the cost of developing new sources of water.

(2) Those persons living in developing countries where protein is produced in insufficient quantities to sustain an increasing population with new demands for better nutrition. The cost and the shortage of fertilizer have severely damaged the "green revolution." Nitrogen and phosphorus of wastewater, all too often rushed out of sight like the proverbial stepchildren, are more and more looked upon as agricultural assets rather than municipal liabilities.

(3) Those persons who fall into the category of concerned taxpayers and those municipal officials who are charged with meeting federal and state water quality standards with precious tax dollars. The beneficial use of domestic wastewater for economic gain is no longer a laboratory curiosity or the practice of poverty stricken foreigners. There are hundreds of projects throughout the United States and thousands in Europe and Asia where wastewater in various degrees of treatment is used for irrigation of forage crops, hardwood and Christmas trees, fish and shrimp culture, powerplant cooling water, factory process water and even recreation. The removal of nitrogen and phosphorus by trees, crops, or fishes not only produces a saleable product but greatly reduces the size and operational budget of mechanical and chemical treatment plants.

The conference and the proceedings that follow have been the result of much thought, planning and effort on the part of persons of many different professional, governmental, and business disciplines. There were 173 participants from 27 states, Canada, Virgin Islands, and Israel.

Many facets of the use of wastewater in the production of food and fiber were discussed in a scholarly, critical manner. The technical and public health constraints were given extra attention.

The success of the conference was largely due to the efforts of Dr. George Allen and his excellent program committee; Charles Newton and his local arrangements committee; Lovena Eaker, my long-suffering secretary; and especially Toni Morrow who wore not only the hat of publicity chairman but that of details clerk, timekeeper, memory bank, bookkeeper, complaint solver, all with a constant smile. To these and Dr. B. Hepher, who keynoted the conference with many constructive comments, I extend my heartfelt gratitude.

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PART 1 INTRODUCTORY SESSION

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OKLAHOMA STATE DEPARTMENT OF HEALTH

REVIEW OF AMERICAN PUBLIC WORKS ASSOCIATION STUDY OF FACILITIES UTILIZING LAND APPLICATION OF WASTEWATER

Richard H. Sullivan
Assistant Executive Director
American Public Works Association

Presented March 6, 1974 at a
Conference on the Use of Wastewater in the
Production of Food and Fiber
Oklahoma City, Oklahoma

REVIEW OF AMERICAN PUBLIC WORKS ASSOCIATION STUDY OF FACILITIES UTILIZING LAND APPLICATION OF WASTEWATER

Richard H. Sullivan, Assistant Executive Director
American Public Works Association

The American Public Works Association, in 1972, conducted an on-site field survey or approximately 100 facilities in all climatic zones where land application of community of industrial wastewaters are being applied to the land, as contrasted to the conventional method of treating such wastes and discharging them into receiving waters. The project was sponsored by the U.S. Environmental Protection Agency under Contract No. 68-01-0732. The report, entitled *Survey of Facilities Using Land Application of Wastewater*, is available from the Government Printing Office as EPA-430/9-73-006 at a price of $6.80.

Additional data were gathered from many existing land application facilities across the country by means of a mail survey addressed to responsible officials. Another survey was carried out to ascertain the nature and extent of state health and water pollution control regulations governing the use and control of land application systems. To augment information on U.S. practices, a survey was made of experiences gained in many foreign countries. In addition, an extensive bibliography was compiled of literature on all pertinent phases of land application practices. The bibliography has been combined with that of three other studies and will be published separately. It will have abstracts of almost 800 articles.

The facilities surveyed were relatively large with long established operations in order that as much operating experiences as possible could be obtained. The surveyed application facilities utilizing community wastes were predominantly located in the western and southwestern portions of the United States, while industrial facilities were generally sited in the northeastern section because this is where the majority of such installations are in service.

Table 1, Summary of Facilities Using Land Application of Wastewater, lists pertinent information concerning the sites for which information was obtained for the states of Kansas, New Mexico, Oklahoma and Texas. Two sites were found in Kansas, nine in New Mexico, three in Oklahoma, and 50 in Texas. Texas and California were the two states with the most facilities. However, in all of the southwest, only two industrial facilities were found and both were in Texas.

Land application of effluent involves many means of accomplishing a variety of objectives. Among the more important objectives, the following were found:

1. to provide supplemental irrigation water;
2. to give economical alternative solutions for treating wastes and discharging them into receiving waters, without causing degradation of rivers, lakes and coastal waters and;
3. to overcome unavailability of suitable receiving waters and eliminate excessive costs of long outfall lines to reach suitable points of disposal into surface water sources.

Among the major means of accomplishing land application of wastewaters are:

1. irrigation of land areas by spraying, with high-pressure or low-pressure devices, of either stationary or movable types of distribution systems;
2. ridge and furrow irrigation systems;
3. use of overland flow or flooding methods; and
4. use of infiltration lagoon or evaporation ponds.

Although facilities of all types were surveyed, the report is primarily concerned with irrigation-type facilities for supplying supplemental water to crop areas, forest areas and unharvested soil cover acreages. The other types are not as widely used, inasmuch as many factors such as climate or soil conditions have an adverse impact on these means of land application.

Table 1. Summary of Facilities Using Land Application of Wastewater By State

State	Information	Population	Flow - mgd	Crops	Area Irrigated Acres
Kansas					
Scott City	Questionnaire	4,325	0.432	Rice	25
Sublette	Questionnaire	1,332	0.13		40
New Mexico					
Almagordo	Visited	25,000	2.5	Alfalfa, corn, oats, sorghum	260
Clovis	Visited	28,000	3.5	Milo, alfalfa, corn, millet, wheat	1,193
Raton	Visited	2,300	0.5	Alfalfa	200
Roswell	Visited	40,000	2-3	Alfalfa, barley, corn, cotton	770
Santa Fe	Visited	45,000	5.3	Alfalfa, apples	740
Lovington	Questionnaire	10,000	0.5		160
Los Alamos Co.	Verified		0.36		
Raton	Verified		0.4		
Silver City	Verified		0.5		
Oklahoma					
Duncan	Visited	20,000	2.5	Wheat, Bermuda grass	180
Boise City	Questionnaire	1,980	0.001		40
Hollis	Verified		0.3		
Texas					
Abilene	Visited	100,000	9.0	Cotton, maize, Bermuda grass	2,019
Dumas	Visited	9,770	1.0	Wheat, maize	585
Kingsville	Visited	30,000	3.0	Maize	606
La Mesa	Visited	11,400	0.6		192
Midland	Visited	62,000	4.3	Bermuda grass, alfalfa, milo, cotton	1,000
Monohans	Visited	8,000	0.8		40
San Angelo	Visited	64,000	5.0	Barley, milo, rye, oats, fescue, alfalfa, Bermuda grass	740
Uvalde	Visited	9,000	0.9	Maize oats	150
Coleman	Questionnaire	5,608	0.3		106
Comanche	Questionnaire	5,000	0.4		12
Cotulla	Questionnaire	3,900	0.165		12
Dalhart	Questionnaire	5,700	0.64	Hay	
Denver City	Questionnaire	4,200	0.15	Cotton	200
Elsa	Questionnaire	5,000	0.18		4.5
Goldthwaite	Questionnaire	1,700	0.2		100
Idalou	Questionnaire	1,800		Feed, cotton	10
Morton	Questionnaire	3,760		Cotton	60
Munday	Questionnaire	1,700			20
Ralls	Questionnaire	2,100	0.1		160
Raymondville	Questionnaire	7,986	0.87		
San Saba	Questionnaire	2,555	0.126		80
Seagraves	Questionnaire	2,500	0.2	Cotton	160
Van Horn	Questionnaire	3,000	0.15		10
Winters	Questionnaire	2,907	0.154	Grain	10
Campbell Soup, Paris	Questionnaire	Industry	3.1		500
Anson	Verified		0.4		
Azle	Verified		0.25		
Crane	Verified		0.2		
Crosbyton	Verified		0.125		
Eldorado	Verified		1.		
Freer	Verified		0.25		
Friona	Verified		<1		
Armour Food, Hereford	Verified	Industry	1.15		
Hondo	Verified		0.4		
McLean	Verified		0.1		

Table 1 (Continued)

Odessa	Verified	1.075
Ozona	Verified	0.3
Petersburg	Verified	0.2
Quitague	Verified	0.06
Rotan	Verified	0.11
Sabinal	Verified	
Santa Ana	Verified	
Seminole	Verified	0.9
Slaton	Verified	0.37
Sonora	Verified	0.3
Stanton	Verified	12
Stratford	Verified	0.01
Sundown	Verified	0.16
Sweetwater	Verified	0.75

Summary of Land Application Sites (Non-Military) For Which Information Was Obtained

	Total Visited	Total Questionnaires	Total Verified	Total
United States	89	122	136	347
Kansas	–	1	1	2
New Mexico	5	1	3	9
Oklahoma	1	1	1	3
Texas	8	17	25	50

Irrigation-type facilities were found to be broadly used under a wide variety of climate and soil conditions, with various degrees of prior treatment of the applied wastewater and various types of ground cover utilized.

Each method of application has inherent advantages and disadvantages which must be evaluated for their feasibility and effectiveness.

The land application of wastewaters has been practiced extensively in many parts of the world for many years with varying degrees of control and success.
degrees of control and success.

As knowledge of wastewater treatment processes improved, and the possibility of confining in a relatively small area the entire process needed to obtain a "treated" effluent for disposal into surface water sources, land application in the United States was relegated in most states to being an undesirable and unacceptable process.

New concerns about preserving the quality and reuse of the nation's water resources have resulted in a reawakening of interest in land application as a viable alternative to conventional wastewater treatment and disposal into receiving waters. Increasing volumes of sewage and industrial wastes, growing complexity of such raw wastes, and mounting needs for water to serve growing urban and industrial processing needs, have created doubts about the ability of receiving waters to assimilate effluents which do not meet high-quality standards. In addition, progressive evidences of eutrophication of non-flowing receiving waters have focused attention on the need to eliminate the presence of nutrients in wastewater effluents. Further, the presence of toxic trace elements in effluents is considered a threat to the safety of waters receiving all but highly purified waste discharges. Thus, advanced treatment methods have been developed and utilized to avoid discharge of such objectionable components.

Inasmuch as land application appears to offer comparable or superior degrees of treatment by augmenting waste treatment with the "natural" purification offered by soil contact, land application is again being considered as one of the acceptable means of achieving full treatment of wastewaters.

However, a most important factor of the current land application concept is that it must be limited to the use of *treated* wastes. Generally, effluents are being conventionally treated to almost meet secondary treatment quality criteria. In at least three observed facilities, applied effluents have received tertiary treatment, to the point where the effluent would fully meet the generally prescribed, as well as proposed, criteria for discharge to receiving waters. Thus, land application is being used to give a degree of advanced waste treatment, including high degrees of nutrient and bacterial removal. In this context, land application can be viewed as an alternative to physical-chemical processes and other methods of ultra-treatment which are designed to achieve a "pure" effluent.

Economics of construction cost, operating costs, energy requirements, and efficiencies of performance of land application systems must be balanced with the ability to acquire the right to apply wastewater upon the required land areas. The cost of advanced waste treatment by conventional means must be weighed in the light of the cost and complexities of land application systems.

Two informative reports were published on the subject of land application in 1972. *Green Lands – Clean Streams,* a report by Temple University Center for the Study of Federalism, is a frankly written advocacy of the land application of wastewaters and sludges. *Wastewater Management by Disposal on the Land,* by the U.S. Corps of Engineers, is a thorough review of the physical, chemical and biological interactions involved in land application.

The firm of Metcalf and Eddy, Engineers, has prepared a companion report for USEPA, concerned with engineering considerations of land application systems. These three reports, together with the report on the study conducted by APWA, should be considered in evaluating land application systems, because they deal with somewhat different aspects of the common problem. The APWA study has made no special effort to examine the specific aspects covered in detail in the other reports. Rather, it is concerned with reporting upon the policies, practices and performances of a representative group of the relatively larger systems within the United States; policies, or lack of policies, of state regulatory agencies; and the experience with land application in some foreign installations.

Systems which were under construction, such as Muskegon County, Michigan, and several major domestic and industrial systems which were intimately known to Metcalf and Eddy project personnel were not investigated for this report. However, Metcalf and Eddy has supplied copies of their field interviews at such sites to APWA for evaluation.

The following highlights from the field surveys are presented in order that a composite picture of observed facilities might be obtained:

1. Communities generally use their land application system on a continuous basis. Food processing plants, the predominant industrial users of the system, generally practice discharge to land systems for three to eight months per year.
2. Ground cover utilized for municipal systems is divided between grass and crops. Industries generally use grass cover.
3. Land application systems are generally used on a daily basis, seven days per week.
4. Application rates for crop irrigation are very low in terms of inches of water per week. Two inches or less was commonly used. (Note: Two inches per week equals 54,300 gallons per acre per week.)
5. Many types of soils were used; although sand, loam and silt were the most common classification given. Two systems using application over many feet of sand were applying up to eight inches per week, and one system on clay was applying only 0.1 inch per day.
6. Most operating agencies, municipal and industrial, are planning to either expand or continue their land application installations. The few examples of systems which

had been abandoned were due to either the desire to make a higher use of the land, or because of reported overloading and poor operation of the land application facilities.

7. Industries surveyed generally treat their total waste flow by land application. Practices of municipalities varied from less than 25 percent to all wastewaters produced.
8. Treatment greater than primary is generally provided by municipalities prior to land application, often times accompanied by lagooning. Industrial systems often treated their process wastes by screening only.
9. Spray irrigation is the most frequently used (57 facilities) method of application, although most municipalities use more than one method. Ridge and furrow irrigation is used at 23 facilities and flooding irrigation by 34 systems. Industry generally used spray irrigation.
10. Land use zoning for land application sites is predominantly classified as farming, with some residential zoning in contiguous areas.
11. Wastewater generally is transported to the application site by pressure lines, although a number of municipalities are able to utilize ditches or gravity flow pipe lines.
12. Many community land application facilities have been in use for several years – more than half for over 15 years. Industrial systems are relatively more recent.
13. Renovated wastewater is seldom collected by under-drains; rather, evaporation, plant transpiration, and groundwater recharge take up the flow.
14. Land application facilities generally do not make appreciable efforts to preclude public access. Residences are frequently located adjacent to the land application sites. No special effort is made to seclude land application areas from recreational facilities and from those who use these leisure sites.
15. Monitoring of groundwater quality, soil uptake of contaminants, crop uptake of wastewater components, and surface water impacts is not carried out with any consistency.

Among the most "advanced" facilities which we reviewed were the Tallahassee system and the systems at Colorado Springs, Colorado, and the University of Pennsylvania at State College.

The largest agriculture venture was found to be at Tula Hidalgo where the wastes of Mexico City, D.F., are applied to the land to supplement the natural irrigation waters of the area. Some 115,620 acres are irrigated and in 1971-72, 1,624,424 U.S. tons of crops were grown. Table 2, Summary of Agriculture production, 1971-72, Tula Hidalgo, lists the 31 major crops which are grown.

Information which was received on crop research conducted at Debrecen, Hungary, is also included in our report. A comparison in yields obtained with the use of treated wastewater as contrasted with normal irrigation practices is given in Table 3, Comparison of Crop Yield, Hungary.

A major concern has also been the boron concentration of wastewater and the relative tolerance of plants to boron. Table 4, Units of Boron in Irrigation Waters for Agriculture Products with Different Grades of Tolerance, was developed by Mexican Agriculture officials. The data contained in the report allows ready identification of where a wide variety of crops have been raised and an indication as to the physical conditions which exist at the site.

In summary, land application of treated municipal effluent and industrial wastes should be a viable alternative to conventional disposal or advanced tertiary treatment techniques in many areas of the country. Full evaluation of application should be made as planning of new or upgraded wastewater facilities is undertaken.

Table 2. Summary of Agriculture Production
1971-72, Tula Hidalgo

Crop	Crop	Hectares	Metric Tons
Alfalfa Verde	Alfalfa	12,396.40	1,181,376.920
Ajo	Garlic	94.50	258.456
Arvejon	Peas (large)	12.89	20.820
Avena Verde	Green Oats	2,998.75	54,426.714
Calabacita	Squash (small)	674.33	7,282.764
Cebada Grano	Barley Grain	1,865.43	3,645.410
Cebada Pago	Barley (forage)		4,059.874
Cebolla	Onion	23.79	168.909
Cilantro (semilla)	Parsley Seed	3.53	4.589
Col	Cabbage	27.95	501.843
Chicharo	Peas	1.00	7.900
Chiles Verde	Green Hot Peppers	768.80	8,231.350
Flores	Flowers	10.41	
Frijol Grano	Navy Beans	1,259.02	1,563.870
Frijol Ejote	Am. String Beans	58.30	151.580
Espinaca	Spinach	0.82	9.020
Frutales	Fruit Trees	25.08	213.180
Girasol	Sunflower	37.19	230.578
Haba	Lima Beans	95.84	1,990.070
Jitomate	Am. Tomato	1,554.65	49,437.870
Lechuga	Lettuce	74.47	1,457.764
Maiz Grano	Corn (kernels)	17,053.60	70,260.525
Maiz Rastrojo	Corn (forage)		65,179.023
Maiz Verde	Corn (sweet)	101.20	7,084.000
Nabo Forraje	Forage Turnips	112.37	1,011.330
Melon	Melon	1.00	7.100
Pepino	Cucumber	34.74	166.752
Pradera	Meadow Grass	12.80	2,080.000
Tomate	Tomato	216.90	2,051.706
Trigo Grano	Wheat Grain	7,293.79	13,865.494
Sandia	Watermellon	0.40	3.960
		46,809.95	1,476,749.371
		Acres	**U.S. Tons**
		115,620.65	1,624,424.30

Note:
1 metric ton = 1.1 (U.S.) tons
1 (U.S.) ton = 0.907 metric tons
1 hectare = 2.47 acres

Note: The crop hectares listed are more than the hectares of land available since a second crop in some instances has been produced on the same land.

Table 3. Comparison of Crop Yield, Hungary

	Debrecen Station (Wastewater)	Gyozelen Farm (Control)
Sugar beets	363.24 q/ha	365.0 q/ha
Dry grain maize	58.5 q/ha	22.49 q/ha
Fodder maize	346.19 q/ha	217/25 q/ha
Alfalfa	99.25 q/ha	46/58 q/ha
Sunflower seed	14.87 q/ha	4.17 q/ha
Industrial potatoes	119.8 q/ha	59.3 q/ha

Table 4. Units of Boron in Irrigation Waters for Agriculture Products with Different Grades of Tolerance

Tolerant	Semi-tolerant	Sensitive
4 ppm	**2 ppm**	**1 ppm**
Asparagus	Sunflower	Mexican Oak
Palm Trees (Phoenix Canariensis)	Potato	Black Oak, Persian or English
	Cotton	
Date (Phoenix Tytiferia)	Tomato	Tuberous Sunflower
	Sweetpea	White Beans
Sugar Beets	Radish	American Elm
Beet		Plum
Betabel	Olive	Pear
Alfalfa	Barley	Apple
Gladiola	Wheat	Grape
Bean	Corn	Fig
Onion	Oats	Nispero
White Radish	Calabash	Cherry
Cabbage	Sweet Potato	Peach
Lettuce	Lima or Kidney Beans	Apricot
Carrot		Blackberry
		Orange
		Avocado
		Grapefruit
		Lemon
2 ppm	**1 ppm**	**0.3 ppm**

Source: Analysis of the Black Waters of the Cuenca of the Valley of Mexico and the Region of El Mezquital, Hidalgo–Bulletin 2 Hydraulic Commission of the Cuenca of the Valley of Mexico, Mexico, D.F., March 1965

WASTEWATER UTILIZATION
IN
INTEGRATED AQUACULTURE AND AGRICULTURE SYSTEMS

by

B. Hepher and G. L. Schroeder*

INTRODUCTION

Israel approaches today a hundred percent use of its water resources. Out of an estimated potential 2.0×10^9 m^3 of water, 1.7×10^9 m^3 are being already used. In these conditions, it is obvious that recycling of wastewater becomes mandatory. The problem, while extreme in Israel, is experienced in many countries.

A semi-arid country, such as Israel, has the highest quantitative demand for irrigation water in agriculture. On the other hand, the qualitative criteria for this water is low. These two considerations make agriculture the prime potential sector for the reuse of wastewater. However, the following limitations restrict this reuse and lower its efficiency:

a) Irrigation is restricted to the dry season, the summer. In winter, there is little use for the wastewater in irrigation. According to a recent study[1], about 5.2×10^5 m^3 of potentially useful wastewater are produced in Israel per day. Of this, in the peak irrigation season 1.7×10^5 m^3, i.e., about 32% are used. However, when calculating on a yearly basis, out of 1.9×10^8 m^3 wastewater produced, only 3.7×10^7, or less than 20% are used. The difference is produced mainly in the winter and disposed of, often in a manner that produces ecological disruption.

b) Irrigation with treated waste effluent is limited by sanitary considerations. Some crops cannot be irrigated by this water because of its residual pathogenic organisms.

c) High salt concentrations make some effluents not suitable for irrigation. The chloride concentration in Haifa sewage, for example, is 480 mg/l (Miron[2]). This limits the agricultural applications to low volumes and a small number of crops.

d) Areas exist where the agricultural demands for water are less than the volumes of treated waste effluent available.

It seems to us that the solution to handling all of these problems encountered in profitably using waste effluent lies in a system which integrates waste treatment with aquaculture and agriculture. The result is a flow of waste from treatment plant through fish ponds to irrigated fields.

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BENEFIT OF FISH PONDS IN WASTEWATER UTILIZATION

Fish culture can accept wider variations in environmental conditions than can agriculture. Fresh water fish can adapt to the high salinities often encountered in waste effluent. The growth of carp, for example, is not inhibited even at salt concentrations as high as 2000 ppm chloride. Tilapia and trout can grow in sea water. The permissible salinity in irrigation water is considered to be 400 ppm chloride, in Israel. Soil quality in fish culture is also less of a limiting factor than it is in agriculture. In Israel, ponds are often constructed on swampy soil and on sand. Water loss by seepage from sand ponds decreases rapidly because of clogging of soil interstices by organic matter. The use of wastewater in fish ponds can come in addition to, or in place of, agricultural use.

It is clear that only impoundment and storage of wastewater effluents can be the answer to the seasonal variations and daily fluctuations in the demand for irrigation water. This impoundment is usually expensive. However, utilizing the impoundments for fish culture cannot only justify the holding expense, but also produce a protein rich food for human consumption bringing an overall profit. It is interesting that the new methods of superintensive aquaculture being developed in Israel are directly applicable to a system integrating usilization of waste effluent and aquaculture. This approach basically combines densely stocked ponds with the introduction of air. The aeration acts as a safeguard against deoxygenation and fish kill. This aeration also can satisfy the uptake of oxygen by the decomposition of the added organic matter. At the same time this organic matter acts as the basic link in the chain of natural foods on which the fish can graze.

The yield of a fish pond depends on the stocking rate of the pond (number of fish per unit area) and the pond management, both of these factors being tightly linked. If the stocking rate alone is increased the amount of natural food per fish will decrease. Fish growth will then decrease or cease entirely and only a small yield or no yield at all will be obtained. However, if parallel to increasing the stocking rate more food is provided, the growth rate is maintained and the pond's yield will be directly proportional to the density of stocking (figure 1). Because the costs of construction and operation of a pond are largely fixed, regardless of the amount of fish stocked, it is clear that the higher the rate of stocking, the greater will be the net profit. The required increase in food to maintain this higher fish density can be achieved in several ways: increasing the production of natural food through the use of chemical and organic fertilizer, in our case waste effluent; better utilization of existing natural foods through polyculture, i.e., the introduction of carefully selected species of fish, each feeding on a different natural food; and, supplementing the natural food with added fish feed.

Supplemental feed is often the largest single operating expense in fish farming. A way should be found to reduce the need for supplemental feed, while at the same time maintaining the high fish yield. This can be achieved by increasing the amount of natural food in the pond. Abundant production of zooplankton in aerated sewage ponds is regularly observed[3]. Benthic fauna are also increased. Wirshubsky and Elchuness[4] found a 30-fold increase in the production of chironomides in ponds receiving organic wastes as compared to non-fertilized ponds. This fauna represents a source of valuable protein readily accepted by fish. We have studied the effect of the introduction of organic matter at high rates on the production of natural foods with fish. Liquid cow shed manure was added to non-aerated ponds with and without fish, at rates up to 100 kg BOD_5 per hectare every two weeks. During winter months (November - February; pond temperature 10°C - 15°C), we observed standing stocks of fauna as follows:

pond type:	no fish manured	no fish no manure	fish manured	fish no manure	
zooplankton	3.3-42.4	<0.055	0.34-1.3	<0.055	gm dry/m^3
chironomides	79-215	1-7	1-4	0-2	100's/m^3

The zooplankton reported are those retained on 150u screen.

The effects of the added organic waste on increasing the standing stocks of natural food are clear. Comparing the data from ponds with and without fish clearly shows the effect of fish grazing on the additional natural food resulting from the added waste.

(1) no fertilizer, no feed; (2) fertilizer, no feed (3) fertilizer, cereal feed; (4) fertilizer, polyculture; (5) fertilizer, protein-rich feed; (6) fertilizer, protein-rich feed, demand feeders; (7) fertilizer, protein-rich feed, polyculture; (8) fertilizer, protein-rich feed, polyculture, aeration

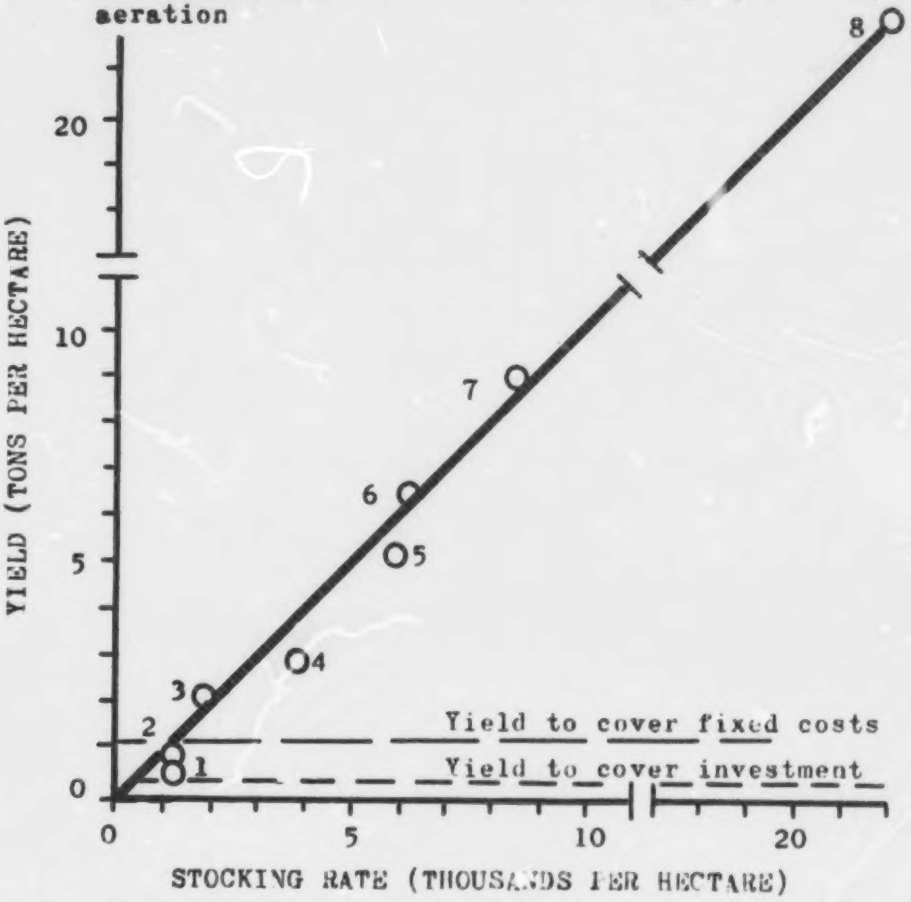

Figure 1. Effect of Stocking Rate on Yield in Fish Ponds

WATER IMPROVEMENT BY PONDS

The sanitary limits placed on irrigation water can be largely overcome by holding the water in fish ponds. A pond, by its very nature, purifies the wastewater by exposure to the high ozygen tension and pH which are produced by phytoplankton. Kisalt and Ilzohöfer[5] demonstrated that the reduction in total number of bacteria in sewage in ponds reaches 99.6%, compared with 90% reduction in activated sludge method, and 89% in trickling filters. We found that the addition of fish to the ponds receiving biological wastes in all instances further improves the quality and increases the capacity of the pond for waste treatment. In the experiments mentioned above, of adding high rates of liquid manure to ponds with and without fish, we observed the following results:

pond type:	no fish, manured	fish;manured	fish;no manure
bacteria (1000/ml)	17 - 27	1.6 - 6.7	0.7 - 4.3
pH (0900 hrs)	7.9 - 8.3	8.3 - 8.9	8.6 - 8.7
D.O. (ppm)	0.7 - 9.5	9.0 - 15.9	10.0 - 13.8
temperature (°C, 0900 hrs)	9 - 15	9 - 15	9 - 15

Bacteria were counted as colonies grown in 0.5% yeast extract agar as described in Standard Methods[6].

One factor which can be attributed to the lower bacteria counts observed in ponds containing fish is the consistently high pH developed in these ponds. Oswald[7] reports an increased rate of disinfection from coliform with increasing pH.

A further benefit of increased pH is an increase in the efficiency with which ponds act as nutrient trap by fixing phosphorous in insoluble compounds and releasing nitrogen to the atmosphere (Hepher[8]; Oswald, ibid). This higher pH appears to be due to the lower CO_2 concentrations observed in the ponds stocked with fish. The lower CO_2, in turn, probably results from lower standing stocks of zooplankton and then bacteria in these ponds, and hence lower respiration volumes.

PROBLEMS WITH EFFLUENTS IN FISH PONDS

Two major factors affect the possibility of using fish in an integrated waste effluent-aquaculture system: dissolved oxygen concentrations in the pond water; and, the presence of piosons, especially detergents, in the waste effluent.

It is clear that the D.O. must be maintained above the lethal level for the species of fish stocked. Even a single case of anaerobic conditions for several hours will result in the loss of the season's entire fish crop. We find that we are able to predict the rate of D.O. depletion in the water by taking into account the BOD of the waste, the pond temperature, and the naturally occurring diurnal D.O. cycle in the pond water. It is clear that in any case these parameters must be carefully monitored. However, having the capacity of aeration as we mentioned above, greatly reduces the hazards of fish kill by suffocation.

Industrial waste may contain lethal concentrations of metallic poisons and must be considered separately. A much more common problem is the piosonous effect of detergents on fish. Hard detergents can pass through a treatment plant with no appreciable reduction in concentration and kill fish. We have worked with waste water from the city of Haifa. This waste often contained about 17 ppm ABS, while the lethal ABS concentration for carp is 10 ppm. In this work we found that even sublethal concentrations reduced the growth rate of the fish. A natural degradation of one ppm ABS per week was observed in the open fish ponds. Based on this our solution was to age the waste in ponds prior to stocking with fish, and then replace daily evaporation and seepage loss by fresh waste. This amounted to an addition of about 100 m^3/hectare/day. The resulting high delution of fresh, by aged, waste made the system operational. With the change to biodegradable soft detergents this problem is greatly reduced.

In considering the problem of integrating aquaculture, agriculture, and waste effluent treatment, we should look at the system as an ecological entity. Fish fill one of the important ecological niches in this system. By so filling this niche we make the system more balanced, more efficient, and more beneficial for man and his environment.

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PART 2 TECHNICAL RESTRAINTS

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DISEASES TRANSMITTED BY FOODS CONTAMINATED BY WASTEWATER

Frank L. Bryan, Ph.D.,M.P.H.

In a world of limited resources and expanding human populations, an ample and safe food supply is vital for populations to thrive. New food sources and improvements in agricultural and aquacultural methods must be investigated and utilized; wastes generated by increasing concentrations of people must be disposed of adequately. Recycling wastewater for watering and nourishing food crops or for growing fish contributes to both food production and waste disposal. But when wastewater is used to irrigate crops or to provide water in aquaculture, appropriate precautions must be taken to prevent the diseases that might otherwise be transmitted by wastewater-contaminated foods.

Foods can become contaminated during production either on farms or in watercourses, during processing in food processing plants, and during preparation in food service establishments and homes. The point at which contamination occurs will depend on the natural sources of a pathogen and on the opportunities for transfer at each stage of the food chain. Factors that contribute to reported foodborne disease outbreaks are reviewed by Bryan[1].

Many of the pathogenic organisms that infect man reach him by being conveyed by more than one vehicle. For example, eggs of some parasitic organisms can appear in feces of infected persons, reach water in which they hatch into a form that infects a vector (as a snail), and, after a period of development metamorphaze into a free-swimming form which penetrates tissues of fish or water vegetables. Diseases caused by such parasites and features of their transmission are listed in Table 1. Few of these parasites are found in the United States, but they are prevalent in some other regions of the world.

Other parasites, such as those that cause hookworm infection, schistosomiasis, and leptospirosis, penetrate human skin in their infective stage and may be acquired by agricultural or aquacultural workers who work in wastewater-contaminated fields or ponds (World Health Organization[2]).

Most of the organisms that are conveyed by wastewater-contaminated foods have less complicated transmission cycles. The organisms that have potential of transmission by such a route, and the diseases they cause, are listed in Table 2.

The following circumstances must occur for persons who ingest wastewater-contaminated foods to become ill:

(1) The infectious agent must be present in citizens of a community or in animals on farms, or toxic agents must be used for industrial or agricultural purposes; and wastes from these sources must reach sewerage or drainage systems.

(2) The agents must survive and pass through all wastewater treatment processes to which they are exposed.

(3) The waste-treatment effluent or watercourse receiving the effluent must be used as irrigation water for crops or as a growing environment for fish or watercrops or for washing or freshening harvested foods. Thus, the agents must survive in the receiving watercourse.

(4) The agents must survive in the soil in which irrigated foods are grown.

(5) The agents must contaminate a food product.

(6) Then one of the following events must occur:

  (a) The agents must be present on the contaminated food in sufficient numbers to survive storage and preparation and still cause illness.

  (b) Bacteria on foods in insufficient numbers to cause illness must multiply and reach levels that are necessary to cause illness.

  (c) Bacteria, and perhaps other organisms, enter food preparation areas on raw foods, where they may be transferred to workers' hands or to equipment surfaces which if inadequately washed will then contaminate other foods that they subsequently touch.

(7) Sufficient quantities of the contaminated food that contain enough of the agent to exceed a person's susceptibility threshold must be ingested. Ingestion of foods contaminated to this level may result in sporadic cases of illness as well as epidemics. When insufficient numbers of pathogens to cause illness are ingested, the infected individuals may become carriers and subsequently contaminate other foods that they touch.

Each step of this chain of events necessary for wastewater to contribute to foodborne outbreaks of human disease is reviewed based on information from engineering and medical literature.

PATHOGENS IN SEWAGE

Numerous investigators have isolated pathogens from sewage. These investigations have been reviewed by Rudolfs *et al.*[3 4], Greenberg and Dean[5], Kollins[6], and Grabow[7]. Animal wastes contain many of the same pathogens as well as other pathogens.

SURVIVAL IN WASTE-TREATMENT PROCESSES

Enteric pathogens survive some stages and sometimes the entire process of wastewater treatment. Table 3 highlights the results of some typical investigations of the effect of various wastewater-treatment processes in removing or killing pathogens. Primary sedimentation usually removes less than 50 percent of coliform and pathogenic bacteria from sewage; it is relatively ineffective in removing viruses and protozoa. Activated sludge or trickling filter processes followed by secondary sedimentation usually removes over 90 percent of coliform or pathogenic bacteria remaining after primary sedimentation. Viruses can be significantly reduced by activated sludge but not by trickling filter processes. Sand filtration is required to remove amoebic cysts and *Ascaris* eggs. Anaerobic digestion reduces 90 percent of pathogenic bacteria from sludge but is less effective for destruction of *Ascaris* eggs. Hepatitis viruses, *Endamoeba histolytica* cysts, and tapeworm eggs withstand the chlorination treatment generally applied to waste-treatment effluent. Thus, many waste-treatment processes remove or kill 90 percent or more of the pathogens that are present in influent sewage, but some still remain in the effluent. If a million pathogens, for example, are present in influent sewage before exposure to processes which remove 90 percent of them, 100,000 survive; in the case of 99 percent destruction, 10,000 survive. Kampelmacher and van Noorle Janssen[22] found that 10^{10} salmonella entered a trickling filter secondary treatment plant each hour and that 10^9 left the plant in the effluent each hour.

SURVIVAL IN RECEIVING WATERCOURSES AND USE OF WASTEWATER FOR IRRIGATION

Those organisms that survive wastewater treatment must also survive in receiving waters. Such survival has been reviewed by Rudolfs[3 4], Dunlop *et al.*[80], Clarke *et al.*[81], Kollins[6], and Grabow[7]. Factors influencing survival in water include temperature, amount of light, flow rate, presence of oxygen, pH, dilution, amount of dissolved material, and types of organisms in the water.

Irrigation water may be sprayed over crops, flow through channels in fields and seep to roots, or be flooded over the field. The numbers of pathogens applied to soil during irrigation can be of such magnitude that outbreaks can result. During an epidemiologic investigation, Renteln and Hinman[176] found that sewage plant effluent used to irrigate a field was channeled by gopher holes to a well pit and entered a community water supply through a defective well casing.

SURVIVAL OF PATHOGENS IN SOIL

After the wastewater or receiving water is used for irrigation, enteric pathogens must survive long enough to contaminate crops. Helminths not only survive in soil but must stay in soil for a period of several days to develop into an infective stage. Survival times for many enteric pathogens in soil are reviewed in Table 4. Factors that effect resistance include number and type of organism, type of soil (structure, moisture content, pH, amount of organic matter) temperature, amount of rainfall, amount of sunlight, protection provided by foliage, and competitive microbial flora. The range of survival times suggests that pathogens introduced into a field by irrigation with wastewater would, despite considerable reduction in numbers, survive in the soil until harvest under some agricultural conditions. Pathogens in soil are more likely to contaminate foods if the soil is kept moist by intermittent application of irrigation water. Also, continuous application of wastewater to soil results in accumulation of pathogens in the soil. Soil filters microorganisms and they often concentrate near the areas where plants grow.

CONTAMINATION OF FOODS

Foods become contaminated from water during irrigation (flooding, spraying, or seepage), from soil when the plant grows, when vegetables or fruits fall on the ground, and when the crop is harvested. They also are contaminated by dust blowing over the crops or from workers, birds, and insects that convey organisms from irrigation water or soil to the foods. Organisms contaminating foods in one of these ways remain on the food surface until they succumb to dessication, exposure to sunlight, starvation, or action of other organisms. They do not penetrate into the vegetables or fruits unless their skin is broken. Survival times of many enteric pathogens on foods are reviewed in Table 5. These times suggest ample opportunity for crops that become contaminated during irrigation or during growth to remain contaminated until harvest. Shellfish and watercress trap and accumulate enteric pathogens; thus, they become particularly hazardous if grown in contaminated waters.

Pathogens are infrequently isolated from foods in fields or after harvesting. When pathogens are found, they are found in a low percentage of samples and usually for only a short time after irrigation. Studies in which isolations were made are summarized in Table 6.

SURVIVAL, CROSS-CONTAMINATION, AND MULTIPLICATION

Foodborne disease organisms must survive processing and preparation steps and bacteria usually have to multiply to reach infective levels. If wastewater-contaminated fruits, vegetables, or seafoods are cooked so that the contaminated portions reach 165°F (or even lower temperatures for sufficient periods of time), vegetative bacteria (but not spores), protozoa, helminthic eggs, and most viruses will be killed. All foods, however, are not cooked before serving, and the temperatures reached during cooking may be too low to kill pathogens.

Contaminated raw foods bring pathogens into food preparation environments. These organisms are killed in foods that are thoroughly cooked, but before cooking they can contaminate the hands of any worker who touches them, or they can contaminate equipment that they contact. Such cross-contamination is commonly reported in outbreaks of salmonellosis; the initial source is usually raw meat or poultry. Some food service workers and homemakers are aware of this danger, but they are unaware that wastewater-contaminated vegetables present the same hazard. Citizens in the United States assume that the fruits, vegetables, and shellfish they eat are free from fecal contamination. But this is the case only if wastewater has not contaminated crops or the waters in which shellfish grow.

Contaminated foods which are to be eaten raw or used as a raw ingredient in a salad can support the growth of foodborne disease bacteria under the following set of conditions: the food must contain sufficient moisture and essential nutrients to support bacterial growth; the foods must be held within a temperature range that permits the contaminating bacteria to multiply, usually near the organism's optimal temperature for growth; and the foods must be held at such temperatures for sufficient time for enough organisms or toxins to be produced to cause illness in those who ingest the contaminated food.

NUMBERS OF PATHOGENS REQUIRED TO CAUSE ILLNESS

Ingestion of contaminated food does not always result in illness. The pathogens must be swallowed in sufficient quantity to exceed a person's threshold of susceptibility if illness is to result. Human volunteer feeding studies have indicated susceptibility threshold levels of various enteric pathogens. Table 7 reviews such studies. Conceivably, wastewater-contaminated food could harbor 10 *Shigella dysenteriae* 1, 180 *Shigella flexneri* 2a, or 1,000 *Vibrio cholerae* biotype ogawa (numbers found to cause illness in adult volunteers) when they reach the consumer. Thus, ingestion of foods contaminated at these levels could result in illness. Such foods could also convey 1 *Endamoeba coli*, 10 *Giardia lamblia*, and 1,000 *Vibrio cholerae* biotype inaba, which if ingested could result in infection and carrier status. Ten thousand *Salmonella typhi* and *Vibrio cholerae* biotype inaba could, conceivably, be present on foods recently fertilized with night soil, human manure, or raw sewage; and ingestion of this amount of these pathogens could cause illness. Certain waterborne outbreaks of these diseases and other diseases, such as salmonellosis, suggest that even lower levels of organisms than those indicated by volunteer feeding studies cause illness. As few as 15,000 *Salmonella cubana* caused death in infants who were given contaminated carmine dye for diagnostic purposes (Lang *et al.*[114]). All volunteer studies cited were conducted on healthy adults. Infants, elderly persons, malnourished persons, and persons with concomitant illnesses would be more susceptible--perhaps a one or more log reduction in dosage could result in illness of such persons. Most of the other organisms in which human feeding tests have been performed, as well as some of the organisms just cited, usually require a period of time for multiplication before the large numbers (100,000 or more) necessary to cause illness would be generated. Thus, it would be unlikely for an infective dose of salmonellae to be on lettuce or other raw vegetables, but it is possible for the same foods to contain an infective dose of shigellae, *Ascaris*, or *Endamoeba* *histolytica*. One saving feature is that shigellae and *Endamoeba histolytica* do not survive long in the competitive environment which occurs in wastewater, soil, or foods. Salmonellae and many of the other bacteria which have been mentioned would become problems if contaminated foods were allowed to stand at room temperatures or refrigerated in large masses.

OUTBREAKS

On some occasions all circumstances described have occurred and outbreaks of human illness have resulted. Such outbreaks and source of contamination and vehicle for each are listed in Table 8. This table, which is obviously biased by incompleteness, shows that foods grown in water have been vehicles frequently. Shellfish which were contaminated in their growing area or during bloating after harvesting were vehicles in 28 outbreaks; watercress was the vehicle in 10 outbreaks; fish were vehicles in 3 outbreaks, and shrimp was the vehicle in 1 outbreak. Vegetables contaminated by night soil, human manure, or raw or partially treated sewage were reported as vehicles in 21 outbreaks. Fruits were considered vehicles in only 4 outbreaks. Before 1960, typhoid fever led the list of foodborne disease outbreaks attributed to wastewater contamination. The foods incriminated in these outbreaks were found to have been grossly contaminated with night soil or raw sewage. Outbreaks of infectious hepatitis are now reported most frequently, followed in frequency by outbreaks of fascioliasis and cholera. These findings reflect, in part, improvement in epidemiologic technique.

The epidemiologic evidence presented in many of the reports would not stand up to critical evaluation. On the other hand, a number of the investigators proved their hypotheses with epidemiologic and laboratory evidence. There are enough of these investigations to indicate that wastewater from mining, industrial, agricultural, community, and household sources have contaminated foods that, when eaten raw, have resulted in outbreaks of foodborne illness. These outbreaks will continue to occur sporadically if raw or partially treated wastewater is discharged into watercourses which are used for irrigation or aquaculture, and the contaminating pathogens survive in the wastewater, in soil, and then on contaminated foods in sufficient quantities.

Table 1. Parasitic Diseases Maintained by the Cycle: Human Feces to Water or Soil; Water or Soil to Food; Food for Human Consumption

AGENT	INTERMEDIATE VEHICLE	INTERMEDIATE HOST	FOOD
Clonorchis sinensis	fresh water	snail	fish
Diphyllobothrium latum	fresh water	copepods	fish
Echinostoma ilocanum	fresh water	snail	snails, clams, limpets, fish, tadpoles
Fasciola hepatica *F. gigantica*	fresh water	snail	watercress, water vegetables
Fasciolopsis buski	fresh water	snail	water vegetables
Opisthorchis felineus	fresh water	snail	fish
Paragonimus westermani	fresh water	snail	crabs, crayfish
Taenia saginata	soil (grass)		beef
Taenia solium	soil (grass)		pork

Table 2. Diseases and Causative Agents Transmissible by Food that Has Been Contaminated by Wastwater or by Soil that Contains Fecal Material

DISEASE	AGENT
Bacteria*	
Arizona infection	*Arizona hinshawii*
Bacillus cereus gastroenteritis	*Bacillus cereus*
Cholera**	*Vibrio cholerae*
Clostridium perfringens gastroenteritis	*Clostridium perfringens*
Enteropathogenic *Escherichia coli* infection	*Escherichia coli* (certain serotypes)
Paratyphoid fever**	*Salmonella paratyphi* A
	Salmonella paratyphi B
	Salmonella paratyphi C
	Salmonella sendai
Pseudotuberculosis	*Pasteurella pseudotuberculosis*
Salmonellosis**	*Salmonella* (over 1,500 serotypes)
Shigellosis**	*Shigella sonnei*
	Shigella flexneri
	Shigella dysenteriae
	Shigella boydii
Typhoid fever**	*Salmonella typhi*
Yersinia gastroenteritis	*Yersinia enterocolitica*
Viruses	
Adenovirus infection	Adenoviruses
Coxsackie infection	Coxsackie viruses
ECHO virus infection	ECHO viruses
Poliomyelitis	Polioviruses
Reovirus infection	Reoviruses
Viral hepatitis**	Hepatitis virus A
Winter vomiting disease	Norwalk agent
Helminths	
Ascariasis**	*Ascaris lumbricoides*
Trichiniasis	*Trichuris trichiura*
Protozoa	
Amebiasis**	*Entamoeba histolytica*
Balantidiasis	*Balantidium coli*
Coccidiosis (*Isospora* infection)	*Isospora belli, I. hominis*
Dientamoeba infection	*Dientamoeba fragilis*
Giardiasis	*Giardia lamblia*

*Other enteric bacteria which could conceivably be transmitted by foods but proof is inconclusive: *Streptococcus faecalis, S. faecium, Proteus* spp., *Providencia* spp., *Klebsiella* spp., *Citrobacter freundii, Enterobacter* spp., *Edwardsiella* spp., *Aeromonas* spp., *Pseudomonas aeruginosa*

**Reported outbreaks, see Table 8

Table 3. Survival of Pathogens During Various Stages of Wastewater Treatment

PATHOGEN	Process								REFERENCE
	SETTLING	ACTIVATED SLUDGE	TRICKLING FILTRATION	ANAEROBIC DIGESTION	SAND FILTRATION	DRYING	STABILIZATION POND	DISINFECTION	
Salmonella typhi		++							(8)
		++							(9)
		++							(10)
		++	++						(11)
	+++			+		-			(12)
							-		(13)
				+		+			(14)
				++					(15)
							+/-		(16)
Salmonella	+	+							(17)
							+		(18)
								-	(19)
						+			(21)
paratyphi		++							(8)
				+		+			(14)
			++						(20)
utrecht	+++		+++						(22)
Shigella flexneri		++							(8)
							-		(18)
Vibrio cholerae		-							(23)
		++							(8)
Streptococcus faecalis			++		++				(24)
	++++		+++					++	(25)

Table 3. Continued

PATHOGEN	Process								
	SETTLING	ACTIVATED SLUDGE	TRICKLING FILTRATION	ANAEROBIC DIGESTION	SAND FILTRATION	DRYING	STABILIZATION POND	DISINFECTION	REFERENCE
Enteroviruses	+	+							(26)
	+	+							(27)
		++						+	(28)
Polioviruses		-							(29)
	+								(30)
	+		+			+			(31)
		+							(32)
								-	(33)
	++++	-	+	-				+	(34)
	+++	++							(35)
	++++	+++							(36)
					-(++)				(37)
	++++	++							(38)
			+++						(39)
							+		(40)
Coxsackie viruses	++++		+++					+/-	(41)
			+						(39)
								+++	(42)
		+							(32)
					+			+	(35)
	++++	-	+	-				+	(34)
	++++	++++	++++		++		+		(43)
			+++						(39)
ECHO viruses		+							(32)
			+++						(39)

Table 3. Continued.

PATHOGENS	Process								REFER-ENCE
	SETTLING	ACTIVATED SLUDGE	TRICK-LING FIL-TRATION	ANAERO-BIC DI-GESTION	SAND FILTRA-TION	DRYING	STABIL-IZATION POND	DISIN-FECTION	
Hepatitis virus A								+	(44)
Endamoeba histolytica	+							++++	(45)
	++++	++++	+++	-	-	+		++++	(46)
	+	+	+	+	-				(47)
						+			(21)
coli	++							++	(48)
Giardia lamblia						+			(21)
Ascaris	+	+	+++	+	-	+			(46)
lumbri-	+	+	+	+	-				(47)
coides	-			+					(49)
eggs	+++							+++	(48)
	+++	++	++	++++					(50)
Trichuris						+			(21)
trichiura	+++	++							(50)
Tapeworm eggs			++++	++				++++	(51)
Taenia	+	+	+	+	-				(47)
saginata	+++	++++	+++	++++	-				(52)

- destruction; + survival; ++ over 90% removal; +++ over 50% removal; ++++ less than 50% removal

Table 4. Survival of Enteric Pathogens in Soil

PATHOGEN	TYPE SOIL	SURVIVAL (in days)	REFERENCE
Salmonella typhi	moist sand	<12	(53)
	peat	<13	
	damp soil	35-74	
	soil surface (sunlight)	5-22	
	soil	70-80	(54)
	soil	35	(55)
	garden soil (in open)	32-58	(56)
	sandy soil	29-36	
	garden soil (hot house)	49-74	
	sandy soil (hot house)	53	
	moist neutral soil	70	(57)
	moist arid soil	<10-30	
	dry soil	<14	
	sand	<5	(58)
	muck	12	
	clay loam	12	
	sandy loam	5	
	adobe	21-105	(59)
	adobe-peat	28-100	
	loam	21-120	
	sand	2-15	
	peat	1-2	
	loam sand	14-60	
Salmonellae	soil and pasture	>200	(60)
	soil (raw sewage)	46	(61)
	soil	46-70	(62)
	sandy soil	35-84	(63)
	soil and pasture	>280	(64)
	soil	147-259	(65)
Streptococcus faecalis	sandy loam	28-77	(59)
	sand	33-35	
	loam	26-63	
	clay loam	33-49	
	muck	40-77	
Endamoeba histolytica	moist soil	6-8	(66)
Ascaris ova	irrigated soil	730-1035	(67)
	soil	2190	(68)

Table 5. Survival of Enteric Pathogens on Foods

PATHOGEN	VEGETABLE	SURVIVAL (in days)	REFERENCE
Salmonella typhi	vegetables (leaves and stems)	10-31	(55)
	radishes	28-53	(56)
	lettuce	18-21	
Salmonellae	vegetables	40	(68)
	vegetables	28	(69)
	beet leaves	21	
	tomatoes (laboratory)	20	(70)
	tomatoes (field)	3	
	soil and potatoes	>40	(71)
	carrots	>10	
	cabbage and gooseberries	> 5	
	tomatoes	3	(72)
Shigella sonnei and *S. flexneri*	clams and shrimp	>60	(73)
	oysters	>40	
S. sonnei	tomato (surface)	2	(74)
	tomato (tissue)	10	
	apple (skin)	8	
Vibrio cholerae	vegetables	5	(75)
	dates	<1-3	
	shellfish	20-45	(76)
	vegetables	7	(77)
	vegetables (refrigerated)	<14	
Endamoeba histolytica	lettuce and tomatoes	<3	(78)
Ascaris ova	lettuce and tomatoes	27-35	(79)

Table 6. Recovery of Pathogens from Foods Contaminated by Wastewater

PATHOGEN	FOOD	SOURCE OF CONTAMINATION	REFERENCE
Salmonella typhi	leaf tips	contaminated soil	(55)
	radishes	contaminated soil	(56)
	lettuce	contaminated soil	
Salmonella (other types)	celery	irrigation water	(82)
	vegetables	irrigation water	(83)
	fish	river water	(84)
	green onions	irrigation water	(85)
Salmonella, shigella, other bacterial pathogens (serologic evidence)	white perch	river water near heavily populated areas	(86)
Enteropathogenic *Escherichia coli*	fish	river water	(87)
Enteroviruses (Polio, ECHO, Reo, Coxsackie)	vegetables	irrigation water	(88)
	oysters	sewage-contaminated sea water	(89)
Ascaris ova	lettuce	irrigation water	(48)
	cabbage	irrigation water	
Helminth ova	vegetables	irrigation water	(90)
	cucumbers	irrigation water	
	tomatoes	irrigation water	
	carrots	irrigation water	

Table 7. Clinical Response of Adult Humans to Varying Challenge Doses of Enteric Pathogens

ORGANISM (strain)	Challenge doses												REFERENCE
	10^0	10^1	10^2	10^3	10^4	10^5	10^6	10^7	10^8	10^9	10^{10}	10^{11}	
Shigella dysenteriae													
(1) (A-1)			+		++								(91)
(1) (M 131)		+	++	+++	++++								(91)
Shigella flexneri (w)										+++			(92)
(2a)					+	+++	++++	+++	++++				(93)
(2a)			+	+++	+++	+++							(94)
Vibrio cholerae													
inaba 569B (unbuffered)					-		-	-	++	++	-	++1	(95)
inaba 569B (+$NaHCO_3$)		-		(+)	+++	+++1	+++1		++1				(95)
ogawa (+$NaHCO_3$)				++			++1						(95)
Salmonella typhi													
(Quailes) vi				-		++		++	++++	++++			(96)
(Zermat) vi					+++								(96)
(Ty2V) vi							++						(96)
(0-901)									++				(96)
(Ty2W)									+				(96)
(Quailes)						+		++		++++			(97)
(Quailes)						++							(98)

Table 7. Continued

ORGANISM (strain)	10^0	10^1	10^2	10^3	10^4	10^5	10^6	10^7	10^8	10^9	10^{10}	10^{11}	REFERENCE
					Challenge doses								
Salmonella newport						+	++						(99)
Salmonella bareilly						+	++						(99)
Salmonella anatum(I)					-	+							(100)
(II)					-	-	-	+					(100)
(III)					-	-	++	+	+				(100)
*							++	+	+				(101)
Salmonella meleagridis(I)					-	-	-	++					(100)
(II)							-	++					(100)
(III)						-	+	++					(100)
*								+++	+++				(101)
Salmonella derby						-	-	++					(99)
Salmonella pullorum(I)					-	-	-	-	-	-	++++		(102)
(II)					-	-	-	-	-	++++			(102)
(III)					-	-	-	-	-	++++			(102)
(IV)					-	-	-	-	-	++			(102)

Table 7. Continued

ORGANISM (strain)	Challenge doses 10^0	10^1	10^2	10^3	10^4	10^5	10^6	10^7	10^8	10^9	10^{10}	10^{11}	REFERENCE
Escherichia coli(O111:B4)							+++		+++	+++			(103)
(O55:B4)									+++	+++	++++		(104)
(O6:H16)									++		+++		(105)
(O124:K72:H-)									+		(+)		(105)
(O143:K?:H-)					(+)		(+)		+++				(105)
(O144:K?:H-)					-		+		+++				(105)
(O148:H28)									+		++++		(105)
Streptococcus faecalis var. liquefaciens									-	+	++		(106)
Clostridium perfringens type A (Heat-resistant)									++	+++			(107)
Clostridium perfringens type A (Heat sensitive)										++++			(108)

Table 7. Continued

ORGANISM (strain)	Feces	Challenge doses 10^0	10^1	10^2	10^3	10^4	10^5	10^6	10^7	10^8	10^9	10^{10}	10^{11}	REFERENCE
Endamoeba coli		(+)	(++)	(++)	-	(++++)								(109)
Giardia lamblia		-	(++++)	(++)		(++++)	(++++)	(++++)						(110)
Norwalk agent (virus) ** 1	++													
2	+++													
3	+													(111)
Hepatitis virus A	+++													(112)
(fecal filtrates)	++													(113)

- = 0, + = 1-25, ++ = 26-50, +++ = 51-75, ++++ = 76-100 percent of volunteers developing illness
* Refeeding trials of volunteers who months before became infected by the same strain
**1,2,3 refers to serial passages of stool filtrates
(+) Infections without illness
1 = cholera-like diarrhea

Table 8. Outbreaks Associated with Foods Contaminated by Sewage or Wastewater

DISEASE	SOURCE OF CONTAMINATION	FOOD	REFERENCE
Typhoid fever	Sewage-polluted waters	oysters	(115)
Typhoid fever	Sewage sludge fertilizer and irrigation	celery	(116)
Typhoid fever	Sewage-contaminated watercress beds	watercress	(117)
Typhoid fever	Sewage-polluted water	shellfish	(118)
Typhoid fever	Sewage-polluted water[1]	oysters, raw vegetables and fruits	(119)
Typhoid fever	Human manure	rhubarb	(120)
Typhoid fever	Privy-polluted watercress beds	watercress	(121)
Typhoid fever	Sewage irrigation	vegetables, blackberries	(122)
Typhoid fever	Sewage irrigation	raw vegetables	(123)
Typhoid fever	Sewage-polluted water	oysters	(124)
Typhoid fever	Sewage-polluted water[1]	oysters	(125)
Typhoid fever	Sewage-polluted water[1]	oysters	(125)
Typhoid fever, gastroenteritis	Fecal-polluted (privy and boats) water	oysters	(126)
Amebiasis	Sewage irrigation	vegetables	(127)
Taeniasis	Sewage irrigation	beef	(128)
Typhoid fever	Sewage-polluted wastes (privies and raw sewage)	oysters	(129)
Typhoid fever, Paratyphoid fever	Secondary sewage treatment (activated sludge)[2] plant effluent irrigation and wash water	vegetables	(130)
Typhoid fever	Sewage	clams	(131)
Shigellosis	Irrigation water (Primary treatment plant effluent)[3]	cabbage	(132)
Amebiasis	Night soil	vegetables	(133)

Table 8. Continued

DISEASE	SOURCE OF CONTAMINATION	FOOD	REFERENCE
Typhoid fever	Sewage-polluted watercress beds	watercress	(134)
Ascariasis	Sewage spray irrigation	vegetables	(135)
Typhoid fever	Sewage manure	vegetables	(67)
Typhoid fever	Night soil	vegetables	(177)
Ascariasis	Night soil	vegetables	(178)
Tyhpoid fever	Sewage irrigation	apples	(179)
Salmonellosis	Sewage irrigation and sludge	vegetables	(136)
Typhoid fever	Human fecal material as manure	endive	(137)
Typhoid fever	Human fecal material as manure	raw salad	(137)
Typhoid fever	Night soil	vegetables	(68)
Ascariasis	Night soil	vegetables	(68)
Ascariasis	Night soil	vegetables	(138)
Hookworm infection	Sewage farming	vegetables	(139)
Viral hepatitis	Sewage-polluted water	oysters	(140)
Typhoid fever	Sewage[1]	vegetables, fruits, shellfish	(141)
Diphyllobothriasis	Sewage-polluted water	fish	(142)
Fascioliasis	Animal feces[1]	watercress	(143)
Viral hepatitis	Sewage-polluted water by primary treatment effluent, raw sewage, and septic tank discharges[3]	clams	(144)
Typhoid fever	Sewage-polluted water[1]	oysters	(145)
Viral hepatitis	Sewage-polluted water (overtaxed treatment plant effluents)	clams	(146)

Table 8. Continued

DISEASE	SOURCE OF CONTAMINATION	FOOD	REFERENCE
Viral hepatitis	Sewage-polluted water (treatment plant by-passed)	oysters	(147)
Viral hepatitis	Sewage-polluted water	oysters	(148)
Viral hepatitis	Sewage-polluted water	oysters	(153)
Fascioliasis	Sewage-polluted water[1]	watercress	(149)
Cholera	Sewage-polluted water	shrimp	(150)
Fascioliasis	Sewage-polluted water[1]	watercress	(151)
Salmonellosis	Dead animal in polluted water from which cows drank	raw milk	(152)
Viral hepatitis	Sewage-polluted water	oysters	(153)
Viral hepatitis	Sewage-polluted water	clams	(153)
Taeniasis	Human feces contaminated trench silo	rare beef	(154) (155)
Minamata disease (organic mercury poisoning)	Industrial waste	fish, shellfish	(156)
Salmonellosis	Sewage-polluted water by untreated sewage (culture positive)	whitefish	(157)
Fascioliasis	Animal feces contaminated watercress bed	watercress	(158)
Viral hepatitis, gastroenteritis	Sewage-polluted water by cesspool	clams	(159)
Gastroenteritis	Sewage-polluted water[1]	clams	(159)
Salmonellosis (Animal infection)	Animal dung slurry used to irrigate pastures	grass	(160)

Table 8. Continued

DISEASE	SOURCE OF CONTAMINATION	FOOD	REFERENCE
Viral hepatitis	Sewage-polluted water[1]	clams	(161)
Fascioliasis	Animal feces contaminated water used for watercress beds	watercress	(162)
Cysticercosis (Animal infection)	Sewage-contaminated irrigation water used for cattle watering	water	(163)
Ouch-ouch disease (cadmium poisoning)	Mining waste used to flood rice fields	rice	(164) (165)
Fascioliasis	Animal feces contaminated watercress bed	watercress	(166)
Viral hepatitis	Sewage-polluted water	clams	(167)
Cholera	Sewage-polluted water[1]	shellfish	(168)
Viral hepatitis	Septic tank effluent contaminated water[3]	watercress	(169)
Salmonellosis (Animal infection,[2] possible human cases)	Human sewage flowing over grazing land	grass	(170)
Viral hepatitis	Sewage-polluted water[1]	clams	(171)
Viral hepatitis	Sewage-polluted water[1]	oysters	(172) (173)
Cholera	Sewage-polluted water for growing and freshening	mussels	(174) (175)
Cholera	Raw sewage irrigation	vegetables	(2)

[1] Implied

[2] Secondary treated sewage

[3] Primary treated sewage

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THE EVALUATION OF MICROBIAL PATHOGENS IN SEWAGE AND SEWAGE-GROWN FISH

by

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A significant removal of pathogenic bacteria and viruses during the process of the lagoon method of sewage treatment is well documented, though not entirely understood[1, 2, 3, 4]. Stabilization ponds with a 30-day retention time can achieve a reduction of up to 99 percent[3]. Okun[5] reported 99.99 percent reduction of coliforms; Drew[6] found 99.6 percent reduction in summer and 96.8 percent in winter.

The use of the traditional coliform test to indicate the likelihood of pathogenic bacteria and viruses has been widely questioned[7] since human pathogens, unlike coliforms, tend to die when not in the temperature and chemical environment similar to a host[8]. The health risks involved in the reuse of wastewater reflect the range of pathogenic organisms found in the community producing the sewage. For example, sewage irrigation of vegetable crops was found to be linked to an outbreak of cholera in Jerusalem in 1970. In India hookworm and gastrointestinal infections are more common among workers on sewage farms than the general population. On the other hand, a follow-up study of the health of workers at sewage treatment plants in the U.S.A. did not reveal any excess risk of disease or disability[3].

Public health officials have noted a mushrooming interest in beneficial uses of wastewater over the past two years. This increased interest is mainly due to the following: (1) the realization that many parts of this country are outgrowing or have outgrown their freshwater supply[9]; (2) the demands for new sources of inexpensive protein to feed an expanding animal and human population; (3) the rising cost of nitrogen and phosphorus fertilizers to support agricultural needs[10]; and last, but not least, (4) the desperate need of municipalities to offset the high cost of meeting the federal and state effluent standards for sewage treatment facilities[11].

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This new surge of interest in beneficial uses of wastewater has prompted the Oklahoma State Department of Health to look objectively at the health hazards associated with human and animal exposure to sewage effluents. Such a preliminary investigation was carried out from July, 1973 through February, 1974 at the Quail Creek Sewage Lagoon System near Oklahoma City. The system is made up of six cells approximately six acres each as shown in Figure 1. It serves a residential district of approximately 10,000 persons producing approximately one million gallons of sewage/day. The first two cells are aerated with the conventional Hinde Air-Aqua System; the four following are operated in series at a level of four to five feet. An engineering flaw in the system is that a modification necessary to convert flow from parallel to series caused a short circuit in cell #4 as shown by the arrows in Figure 1.

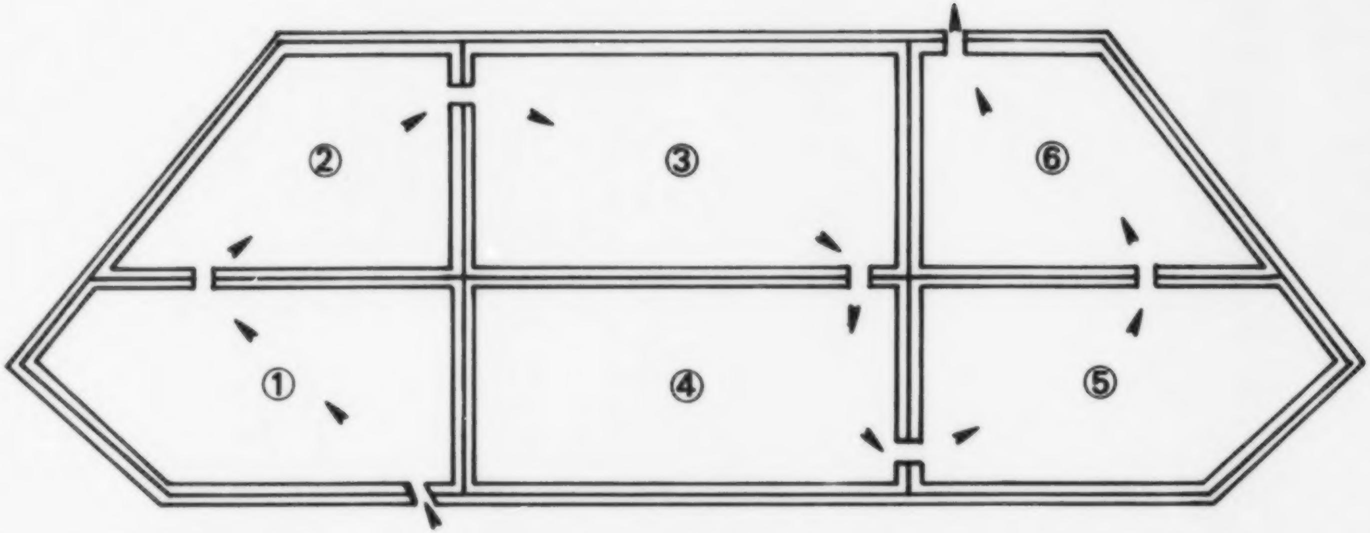
In May, 1973, 25,000 two to four-inch channel catfish fingerlings were stocked in each of cells #3 and #4; 35 pounds of adult Golden Shiners (Notemigonus crysoleucas) were stocked in each of cells #5 and #6. In July, 175 three-inch Tilapia nilotica and five pounds of Fathead Minnows (Pimphales promelas) were introduced into cell #3 only.

Despite what was considered as adequate screening between the test cells which were dry when the study began and the proceeding two conventional cells, wild fish including Black Bullhead (Ictalurus melas), Green Sunfish (Lepomis cyanellus), and Mosquitofish (Gambusia affinis) contaminated the test cells.

MATERIALS AND METHODS

One hundred seventy-nine fish on 12 different dates and 77 water samples on 11 different dates were collected over a seven-month period beginning July 1, 1973 and ending January 28, 1974. While there was some variation, most water collections consisted of four liter catch samples of the untreated sewage and similar samples of the effluent from each of the six cells. A total of 34 samples of five channel catfish each were collected from cells #1 through #5 during 12 fish-sampling visits. Tilapia, Green Sunfish and Bluegill were collected one time each from cells #3, #4 and #5 respectively. Water samples were processed according to the "APHA Standard Methods of Examination of Water and Wastewater."[12]

Water samples were examined for total coliform, fecal coliform, fecal streptococci, and pathogenic enteric bacteria using the membrane filter methodology recommended by the APHA[13]. For isolation of pathogenic enteric bacteria, 100 ml portions of sample were passed through 0.45 micron pore size membrane filters. The membranes were then placed in Hajna's gram negative (GN) enrichment broth and incubated overnight.



QUAIL CREEK LAGOON SYSTEM

FIGURE 1

The broth was then streaked onto Bismuth Sulfite Agar, Brilliant Green Agar, XLD Agar, and MacConkey's Agar. Suspicious colonies were confirmed by conventional biochemical and serological tests as described by Edwards and Ewing[14]. GN broth was initially selected for its known superior ability to yield shigellae species while it is also reported to be suitable for enrichment of Salmonella species[14, 15]. Failure to isolate Salmonella from the first nine samples examined led to the addition of a second membrane which was enriched in Tetrathionate broth with Brilliant Green added[13]. This broth was then used to streak additional plates of Brilliant Green and Bismuth Sulfite Agars.

Subsequent control studies in our laboratory showed that Tetrathionate broth with Brilliant Green added can recover essentially all Salmonella seeded at low levels into lagoon water while, by comparison, the yield from Hajna's GN broth was reduced by a factor of greater than ten.

For virus studies, 50 ml aliquots of lagoon water were centrifuged at 2,000 rpm. The supernatant was decanted, treated with antibiotics and 0.1 ml portions inoculated into Rhesus Monkey Kidney, Wi38 and, when available, HEp2 tissue culture tubes. Cultures showing cellular destruction were examined by conventional methods for viral identification[15]. Cultures not showing cellular destruction were passed once to the same cell line before discarding as negative.

It was recognized that the catch sample method has a comparatively low degree of sensitivity; but the elaborate concentration techniques employed by Wallis, Melnick and Fields[17, 18] and Metcalf, Vaughn and Stiles[19] were beyond the financial scope of this study. Control studies conducted by seeding of the lagoon water with low levels of Coxsackie A9 enterovirus followed by the above procedure showed no apparent interference to the recovery of virus by naturally occurring constituents in the lagoon water. Samples of fish were carefully dissected and the skin, upper intestines, cloaca, and muscle were cultured separately. However, after the first two collection dates, skin was combined with muscle to constitute one specimen, and, all viscera were pooled to constitute a separate specimen. After homogenizing, an aliquot of each pool was used for both virus and bacterial study.

For isolation of human pathogenic enteric bacteria, a 2 ml portion of the homogenate was enriched by overnight incubation in 10 ml of Hajna's GN broth followed by streaking for isolation as indicated previously for water. Virus examination was accomplished by processing the homogenate according to the procedures in use for human feces and tissue by the Oklahoma State Department of Health Laboratory[16]. The processed material was assayed by inoculation into Rhesus Monkey Kidney, Wi38 and, when available, HEp2 tissue culture systems.

Results of the study are shown in Figure 2. The log count of fecal coliforms per 100 ml is shown in the bars and sampling dates are shown along the horizontal axis. The top shaded area shows the log of number of coliforms in the raw influent to the system and the lower portion of the bars, shown by the dotted area, represents the treated effluent as it passed out of the last of the six cells into the receiving stream.

Inspection of these bars readily shows that there is a remarkable reduction of fecal coliform bacteria to less than detectable limits in a standard 50 ml portion in three separate samples.

The pathogens isolated are shown by the boxes at the bottom:

August 13 there was an isolation of Edwardsiella tarda.

December 17 there was an isolation of ECHO 1 enterovirus.

January 14 there was an isolation of Salmonella newport.

Figure 3 shows the average log of the count of indicator organisms per 100 ml isolated on the 11 water-sampling dates. As the wastewater flows through the system from raw influent on the left to effluent from the last lagoon on the right, one can see a progressive reduction in the indicator organisms. By the time the effluent passes through the third cell, it is found to contain less than ten fecal coliform organisms/100 ml, and remains at this low level as the effluent passes through the last three cells. It is interesting from a public health standpoint that of the three pathogens isolated in water during the study, two were found in raw influent and one was in cell #2. No pathogens were found in wastewater beyond the first two conventional cells; nor in any of the cells containing the test fish; nor in any of the 179 fish sampled.

In conclusion, it can be said that this study has confirmed the previous observations of others that indicator coliform organisms are efficiently removed in a lagoon-method wastewater-treatment system[1, 2, 3, 4]. This study has shown that human pathogens are rare in those wastewaters tested and in fish grown in those wastewaters beyond the raw or first two conventionally-operated cells.

Though it was not within the scope of this study to evaluate the effect of extended lagooning, per se, on removal of coliforms, future investigators should design studies in such a way as to simultaneously compare the results in lagoons both with and without the addition of fish.

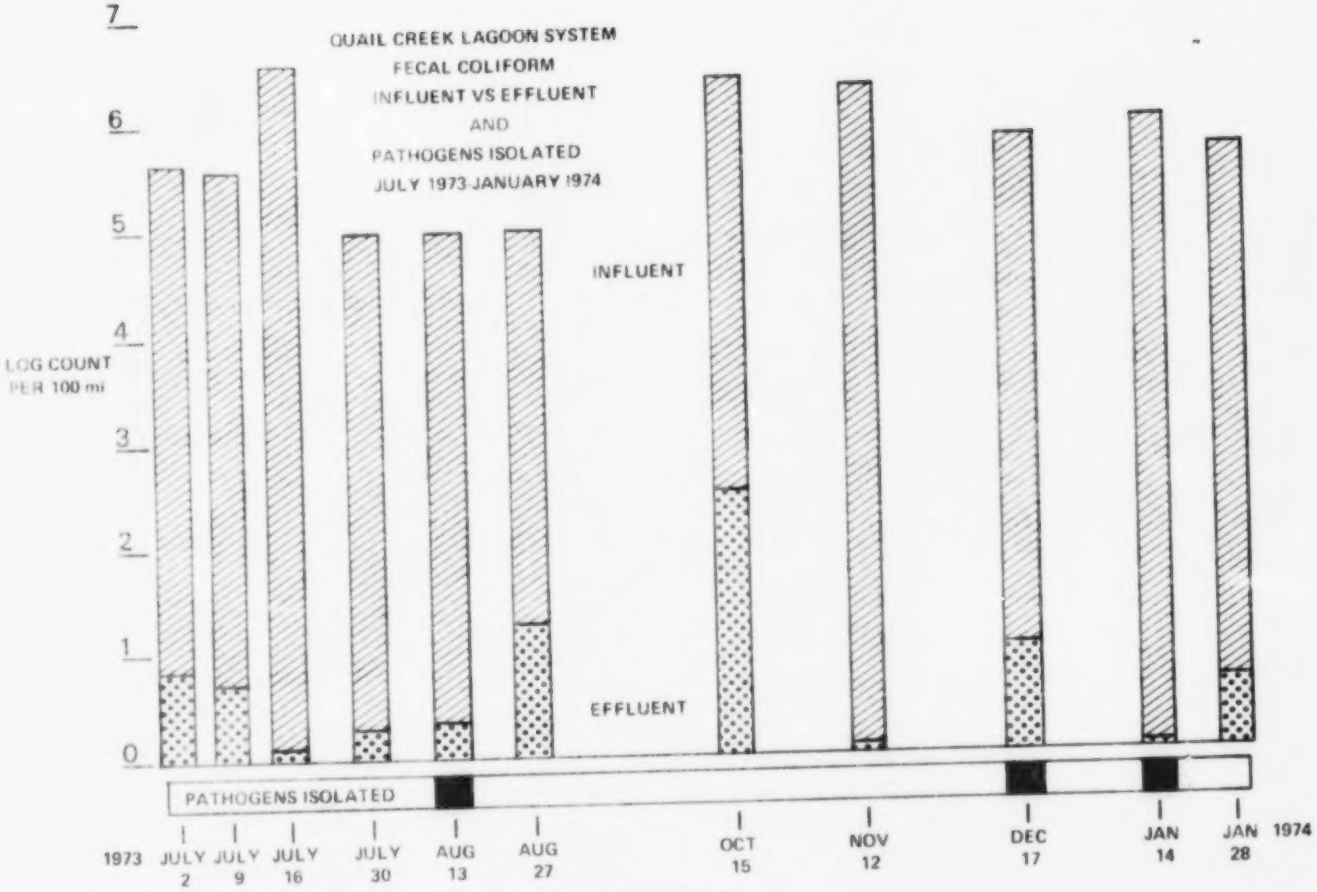

QUAIL CREEK LAGOON SYSTEM
FECAL COLIFORM
INFLUENT VS EFFLUENT
AND
PATHOGENS ISOLATED
JULY 1973-JANUARY 1974
LOG COUNT
PER 100 ml
7
6
5
4
3
2
1
0
INFLUENT
EFFLUENT
PATHOGENS ISOLATED
1973
JULY 2
JULY 9
JULY 16
JULY 30
AUG 13
AUG 27
OCT 15
NOV 12
DEC 17
JAN 14
JAN 28
1974

FIGURE 2

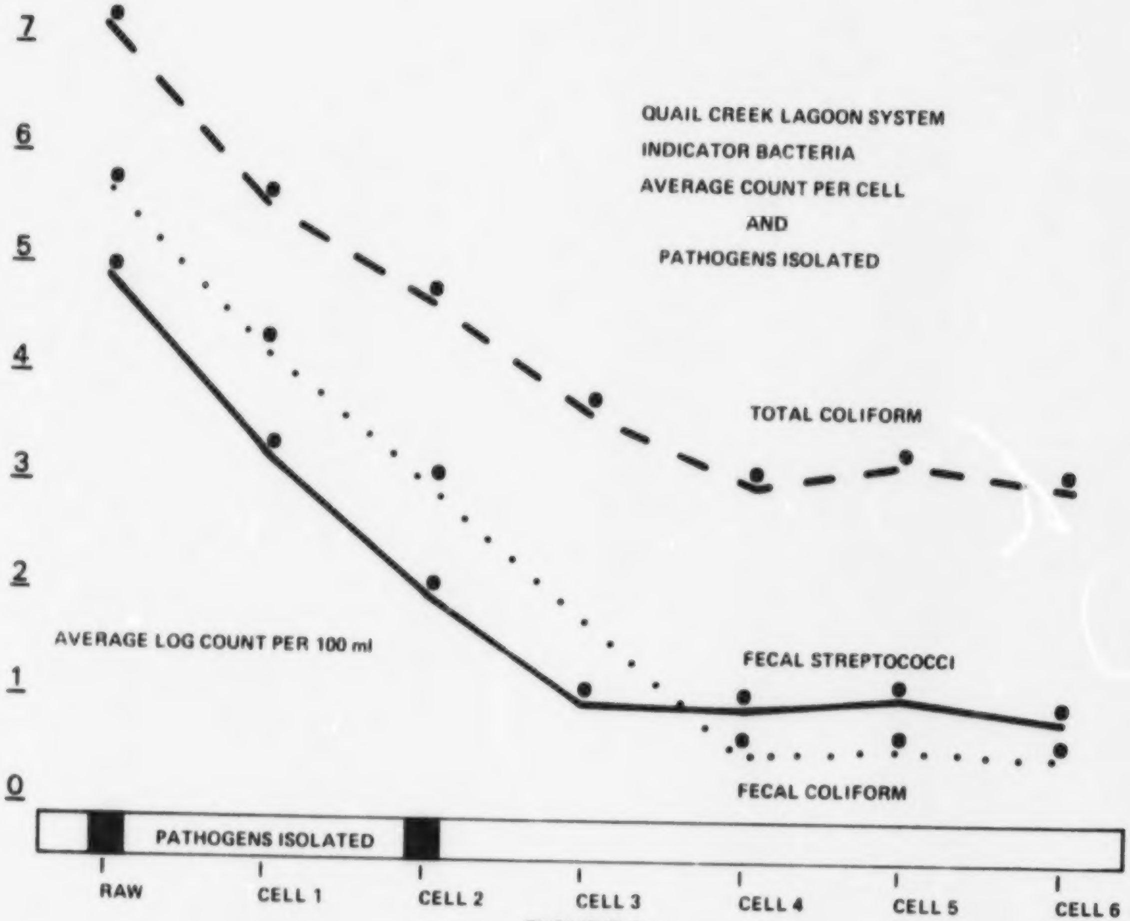

QUAIL CREEK LAGOON SYSTEM
INDICATOR BACTERIA
AVERAGE COUNT PER CELL
AND
PATHOGENS ISOLATED
7
6
5
4
3
2
1
0
AVERAGE LOG COUNT PER 100 ml
TOTAL COLIFORM
FECAL STREPTOCOCCI
FECAL COLIFORM
PATHOGENS ISOLATED
RAW
CELL 1
CELL 2
CELL 3
CELL 4
CELL 5
CELL 6

FIGURE 3

Many economic and conservation-oriented pressures are prompting a surge of interest in using domestic wastewater for production of crops and fish. This study supports this concept. But, as Shuval so aptly pointed out, much work yet remains to be done in accurately determining the public health hazards associated with such practice[20].

We should investigate the potential dangers to the handlers, harvesters and processors of fish and shellfish grown in wastewaters and not limit our attention to the consumer only. We must sharpen our techniques for isolating pathogenic viruses and bacteria from wastewaters, use them more frequently in our monitoring programs and not rely solely on coliform indicators as we have in the past. Parasites such as worms and amoebae should not be overlooked. And last, where possible, we should use sound epidemiological methods to correlate the presence or absence of disease in a community with those organisms found in that community's wastewaters.

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MORBIDITY RISK FACTORS
from
SPRAY IRRIGATION WITH TREATED WASTEWATERS

by

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In this paper we present the interim conclusions of an ongoing literature review. We have tried to find out whether properly treated wastewaters from a properly designed and correctly operated wastewater treatment plant, which provides at least secondary treatment, are safe from the health viewpoint. In particular, we wished to find out whether aerosols generated from these treated wastewaters present a significant pathogen risk. If not, we believe, their use on land for irrigation and for their fertilizer value can and should be encouraged for a variety of environmental, economic, agricultural, and social reasons.

In our search we have adopted the antagonist attitude. We have sought to prove to ourselves that there are valid reports which show that disease has occurred from this practice in the past, and therefore that the practice must be controlled.

Since we have not yet found any such reports, we continue to recommend that the many advantages known to accrue from this practice outweigh the apparent chances of a health risk, and that the practice should be encouraged. We will now show our reasoning, based on the findings we have examined so far.

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FINDINGS

Raw Night Soil

There is no room for doubt that, in an area where an enteric infection is endemic in a human population, one important factor by which endemicity is maintained is the practice of applying fresh raw sewage as a fertilizer on to crops which are eaten fresh and raw by man.[1] There is continuing discussion whether this same practice helps to maintain resistance to the disease in the resident population. But that is irrelevant to the argument that the practice transmits disease to visitors, and therefore is a public health risk.

We have not yet found documentation whether pulmonary infection can be spread in this way. There is published evidence that bathing in surface waters which are contaminated by human fecal matter may have caused disease.[2]

Urban Wastewaters

In urbanized communities where the flushing toilet and sewer system are in use, the human waste load is subjected to a dilution of some 200-2000 times - or more, if the storm drains are combined with the wastewater sewers. Further, in properly sewered urban societies, the endemic morbidity, and hence the average pathogen load per person, is reduced by a factor of some 10-100 times. These two factors combined produce a total pathogen reduction per unit volume of between 2 x 10^3 and 2 x 10^5 when raw modern city sewage is compared with raw night soil applied to crops or to land. A further reduction in viable pathogen load from urban sewers is caused by the extended time-delay during transport from source to crops.

Some cities have continued or have re-introduced[3] the 'sewage farm' procedure first practiced in the late 1800's. In this technique the raw sewage is applied year after year to a limited area of land, which is used for crops or for pasture. In spite of the large quantities which have routinely been applied, in some cases over many years consecutively, there are no recent reports of disease resulting.[4] Indeed, one such city reports that the carcasses of cattle grazed on this land attract better-than-average prices in the market because of their higher quality.[5]

Sedimentation - It is generally accepted that treatment by primary settling reduces the general pathogen load in the supernatant waters. There is evidence that primary settling carries most of the viral load into the sludge, probably by adsorption;[6] this effect is accentuated by chemical flocculants;[7] the degree of inactivation of this virus load is not yet agreed. The supernatant waters retain a significant proportion of the initial nonviral pathogens, and the balance settles with the sludge. The ultimate degree of any reduction . . . .

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of the total initial load depends on the time delay during travel in the sewers and in the settling system, and can be attributed to normal die-off, possibly accentuated by the bacterial activity of metabolic byproducts of non-pathogenic micro-organisms.

Aerobic Treatment - It is generally accepted that properly conducted aerobic treatment, whether by biological filter or by activated sludge tanks, in a plant which is not being overloaded or mismanaged, can reduce the non-viral pathogen load by better than 99%, although this is seldom achieved in practice.

When the effluent waters are properly chlorinated to the required standards, they are generally accepted to be safe for discharge into a waterway when judged by the criteria in force. There is satisfactory evidence that such waters are acceptable to the public for irrigation of agricultural soil, parkland, and golf courses,[8] since several states have set criteria to regulate this practice.[9,10] Indeed, in many areas today the more expensive developments, which include a golf course as one of their amenities, are routinely requesting permits to irrigate their courses with the effluent from their treatment plants.[11] We have found no evidence of disease reported from this practice.[12]

Anaerobic Sludge - While there are reports of disease being acquired from sludge,[13] it is likely that these may have been primary settled sludge, since the majority of treatment plants in the U.S.A. are still confined to this simple procedure.

There is satisfactory evidence that secondary anaerobic treatment of sludges, properly conducted in a properly designed plant which is not being overloaded, produces a sludge which is acceptable as safe for public use as a soil improver without further disinfection or sterilization. This sludge has been applied wet (fresh) onto farmland and on to city parkland[14] and children's playgrounds.[15] We have found no instance of reported disease from this practice.

Further, such sludge, after drying, is saleable[16] as a profitable venture over long periods of time both retail[17] and contract[18] when properly marketed. We have found no instance of reported disease from this practice.

Irrigation - We have found no instance of disease reported from spray irrigation with chlorinated secondary effluent that has been properly treated ('green water'). This is not surprising. If pathogens were a risk from drift, spray, or aerosols generated from sewage, surely the clinical risk would be greatest where the concentration is greatest - in the sewage works, near the aerobic treatment unit? We have found no evidence that long-term or newly introduced operators have disease attributed to this; indeed some experts have suggested that the morbidity of sewage plant operators is less than the morbidity of the general public. Perhaps a more sensitive indicator could be the adolescent school children visiting the sewage plant as a science course field trip; a letter from the National Science Teachers Association tells us that "From inquiries to the U. S. Public Health Service and the Montgomery (Maryland) Health Department, we have learned of no reported illnesses to students following visits to sewage treatment plants, nor could we find that any guidelines have been developed or are in practice as precautions for such visits. Also, the student tours director for the Washington Suburban Sanitary Commission told us that no special precautions have been observed in their tour program, nor did he know of any illnesses to students following tours of that facility."[19]

CONCLUSIONS

The Published Evidence

In short, our review of the literature has not yet provided an instance in which satisfactory evidence is given that a disease incident has arisen from using treated sewage products. By treated we mean that the evidence provided shows that during the days preceding the incident: the treatment plant was operating within design capacity; that the unit processes in that treatment plant were being operated properly and were functioning properly; and that the effluent quality was within the criteria required. In other words, we suggest that before an incident can justifiably be attributed to the use of treated waste waters, it must be shown that those wastewaters were being fully and properly treated during the period of time which could have been implicated. Until that has been demonstrated one would be calling in question not the health risks of using these materials, but the effectiveness of the design and the operation of that plant. And that demonstration should not be difficult in the future, even though it may, on occasion, have been so in the past.

THE MAGNITUDE OF THE RISK

Infectivity

In actual practice, what is the likely risk that pathogens originating in a sick person or a carrier will gain entry to a susceptible person? In the U.S.A. the pathogen endemicity load is now low. Will the combined effects of attrition by die-off over time, of dilution into a large volume of water, of attenuation by spread over a large area of land, of sterilization by solar radiation, and of degradation by soil organisms - will all these effects, working in series, reduce the real risk to below the level of risk in a city street[20] to below that of the risk from food obtained from commercial sources,[21] or to below that in an office building? We can suppose so, but this also requires evidence, and we have not yet found that in the literature.

Spread - Much has been published about the effect of the configuration of the spray-nozzles of fuel injectors for reciprocating and for jet engines, on the size distribution of the droplets produced. There is also information about the design of applicators for agri-drugs in the field, and about the factors concerned in reduction of drift and of wind spread: the smaller the droplet, the further it travels; to reduce drift, droplet sizes should be larger than 500 microns.[22] There is literature which suggests that droplets often nucleate around a bacterium; and that the physical changes which occur in the droplets during a short time after its formation are complex: these include relative change of concentration of solutes across the diameter, and dessiccation; it may be speculated that these changes may be so fast as to produce effects comparable to freeze-drying, and so may preserve a pathogen rather than destroy it. The free-floating spherule so formed behaves like a dust particle. Droplets and dust can travel a considerable height into the atmosphere before coming down again,[23] or they may be blown long distances near ground-level before reaching their final resting-place - 50 miles has been recorded for an aerosolized agri-drug.[22] These effects, recorded in the literature from other specialties, require to be related to our questions.

Susceptibility - If we assume that a pathogen has survived these ordeals and has gained entry to a human being, what are the chances of its causing an infection? Will that infection be sub-clinical and cause no apparent loss of health? and if so will it produce another carrier? Or will it cause recognizable disease which will affect the patient or society adversely? There is evidence that mere entry is not always sufficient to start an infection; the person has a battery of defense mechanisms, and the entrant pathogen is more likely to be destroyed than not. But there is evidence that although it requires many bacteria to produce infection,[24] a single viral $TCID_{50}$ can do so if it achieves a susceptible target - for example, the lung alveolus; in this instance small droplet size (below 2.5 microns diameter) is a required factor.[25] There is agreement that although bacteria and perhaps parasitic cysts can confidently be removed by conventional sewage plants the same cannot be said with the same confidence about viruses.

NEED FOR INFORMATION

We can summarize as follows:-

1). A literature search has not yet revealed any incidence of disease from irrigation with properly treated sewage products.

2). Irrigation techniques should strive to eliminate the formation of any droplets that are smaller than 500 microns in diameter.

3). Researches should focus on determining the viability of pathogenic organisms through the several attenuation pathways to which they are normally subjected, so that we can quantify the actual risks of infection. Then we can work to minimize those risks if they prove to be significant.

DISCUSSION

We recognize that in this paper we have posed more questions than we have provided evidence or answers - and properly so. It has been our purpose to reveal those questions, and so, hopefully to cause the needed investigations to start.

Nevertheless, the existing information does not warrant any restrictions on the use of properly treated wastewaters for land application; but it does support a case for careful management of sewage plants whose products are to be used in this way. Both the better management of wastewaters, and the greater use of the treated end-products are commended by government and professional[26] agencies as a national goal; they are therefore likely to be achieved soon. Our investigations so far confirm the need for both.

This brief paper is not claimed to be exhaustive, nor are our findings conclusive: it is designed more to share with you the large areas of doubt that exist both for and against there being a significant pathogen risk.

At the International Conference on Land for Waste Management held in Ottawa last October, a group of conferees came to the following conclusions:-

1. NEED FOR COORDINATED INFORMATION CENTER
Nobody present knew of a coordinated bibliography or information reference center which meets the present needs of administrators, field workers, and research workers, about the pathogen risks from use of treated wastewater products. This need is growing.

2. NEED FOR COORDINATED RESEARCH
The information so far gathered by those who were present is inconclusive and/or inadequate to support policy recommendations or decisions at field, administrative, or guideline legislative levels. Basic research is urgently needed. Over-restrictive legislation based on caution might soon be introduced which could obstruct trial of innovative procedures unless such basic information quickly becomes widely available.

3. SCOPE
These information deficiencies apply to concentration and/or dissemination of pathogens from municipal sewage treated effluents, from landfill operations, from resource recovery systems, and from animal wastes. Information from these sources is also lacking about the health of the operators who are directly working with the materials and processes, and of the health or disease implications for the public at large, for domestic animals, and for game and wildlife; furthermore, concentration and/or dissemination of plant pathogens should also be included in the enquiry.

Bibliography

It was agreed that it would be desirable to set up a central reference bibliography, and to provide the information as required. That bibliography is now being compiled from information still being sent in. You are invited to join in the endeavor by sending in the citations of your own bibliography, preferably with your annotations or reviews. Sending your citations is one certain way of ensuring that you will receive copies of the compilation.*

Research Program - Future research may well be conducted under the following headings:-

1. Evidence of morbidity from aerosols that have originated from treated effluent wastewaters, established under the criteria we have suggested.

2. Information on the travel of aerosols, and their spread or capture under field conditions.

3. Information on the survival of pathogens in aerosols, and of their infectivity after reactivation.

4. Environmental factors that perturb these prime findings.

Only when we have a substantive body of information on at least those points will we be able to proceed to the real tasks before us:

5. Evaluation of the risks; and, finally

6. Preparation of recommendations, guidelines, and model codes.

A program of this magnitude can only be accomplished by numerous teams working together to an agreed schedule. It is our hope that the information flow into and from this study group will encourage that co-ordination; we welcome your comments.

This paper is a summary of one section of a larger three-part work presently in preparation. At this stage it must be regarded as an interim draft, which we have presented today for your comments and improvements.

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PERMISSIBLE LEVELS OF HEAVY METALS IN SECONDARY EFFLUENT FOR USE IN A COMBINED SEWAGE TREATMENT-MARINE AQUACULTURE SYSTEM I. MONITORING DURING PILOT OPERATION[*]

by

W. B. Kerfoot and S. A. Jacobs

Domestic wastewater inherently contains a higher load of metals than the original source water due to the treatment of water for algae control, leeching from pipes, and solutes added during household use[1,2]. While nutrient-rich secondarily-treated wastewater can serve as a fertilizer for aquaculture of commercially valuable algae and shellfish[3,4,5], excessive levels of metals in solution may be toxic to the cultured organisms, accumulate in the meat of food products to an extent to pose a danger to public health if consumed, or impair the visual appeal and taste of the meat. The question arises, does the elevated metal content of a secondarily-treated wastewater render it unsuitable for aquacultural purposes? If not, what levels of heavy metals in the effluent are acceptable for aquacultural usage since domestic effluents from industrialized regions tend to contain more elevated concentrations than those from rural sources[6]?

Within this paper we address the first question, citing the levels of cadmium, lead, copper, zinc, chromium, and nickel observed in the effluent, seawater, and oysters during operation of a pilot sewage treatment-marine aquaculture system. Guidelines for acceptable levels of metal in the effluent for use in an aquaculture system are developed later in the companion paper.

THE WASTEWATER TREATMENT-MARINE AQUACULTURE SYSTEM

Secondarily-treated wastewater, diluted with seawater, makes an excellent medium for growing marine phytoplankton. The phytoplankton is, in turn, removed from the water by filter-feeding shellfish, converting the organically-fixed nutrients into valuable protein (figure 1). Nutrients regenerated directly from the excretion or indirectly from faecal deposits of the shellfish are removed by the growth of macroscopic algae in tanks before discharge of the effluent to coastal water.

As described in detail previously by Ryther *et al.*[3] and Goldman *et al.*[7], the system has several distinct advantages over previous

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algal processes for nutrient stripping. First, removal of algal biomass is accomplished by using the algae as a food source rather than an end product which must be disposed of. Secondly, the herbivores represent a highly desirable, and economically important protein source. Thirdly, the process lends itself to the development of a multi-component food-chain system useful for the production of many commercially valuable food crops. For instance, a town of 50,000 people with a 126 acre aquaculture-sewage treatment system could raise an annual crop of over 900 tons of oyster meat, worth in today's market upwards of 5 million dollars as a luxury table oyster or 1 million dollars as canned or frozen products.

The design of the system is fairly simple. Effluent is added to filtered seawater at a ratio of 1:4 (figure 1). Phytoplankton cultured in this mixture flows into tanks of shellfish where it mixes with diluting seawater at a concentration of 1 part culture to 19 parts 100 µ-filtered seawater. The remaining water flows full strength into the macroscopic algae tanks and then, finally, is returned to the coastal waters. Other ratios of effluent:seawater ranging from 1:10 to 1:0.5 and ratios of algae culture:seawater ranging from 1:1 to 1:10 have been used. The above conditions applied to experiments performed during summer 1972 and winter 1973.

During the operation of the pilot project, the dominant species in the culture vats were always diatoms - usually either *Phaeodactylum tricornutum* or *Chaetoceros simplex*, often interspersed with smaller numbers of assorted diatoms, flagellates, and green algae. The oyster culture system was a 760 liter rectangular tank (265 cm long, 62 cm wide, 53 cm high), constructed of fiber glass-coated wood. The oysters, numbering 500 in total, were positioned on trays of Vexar mesh. Overflow from a standpipe in the shellfish tanks was introduced into another rectangular wooden tank which held seaweed (*Chondrus crispus* and *Ulva lactuca*) for final removal of nutrients from the water before discharge.

TRACE METALS IN THE SYSTEM

The metals selected for this study are capable of being concentrated in the tissue of marine organisms: cadmium, which has been shown to cause kidney failure in humans and the "Itai-Itai disease" if present in sufficient quantities in consumed products[8]; lead, which may cause encepholopathy, anemia, and renal damage if present in high concentrations in meat[9]; copper, which may be toxic to algae[10], lead to discoloring and bitterness of oysters[11], or may be toxic to human consumers if present in high concentration in the meat[12]; zinc, which may interfere with growth or organisms in the culture system if present in high enough concentrations[10], but does not present a danger to consumers[13]; chromium, which may be carcinogenic at elevated concentrations in food[13], and nickel, which may be toxic to algae and fish[14] but whose effect on consumers of meat is uncertain[13].

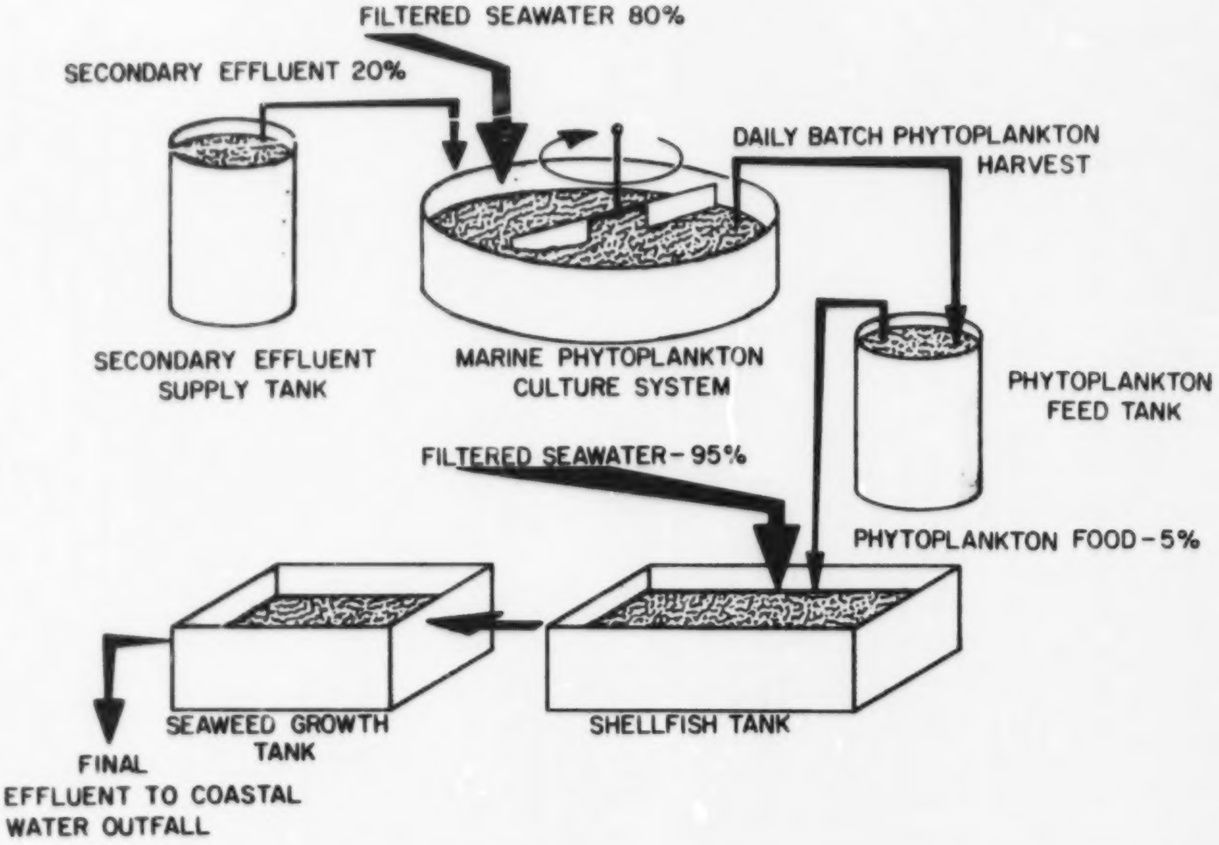
FILTERED SEAWATER 80%
SECONDARY EFFLUENT 20%
DAILY BATCH PHYTOPLANKTON HARVEST
SECONDARY EFFLUENT SUPPLY TANK
MARINE PHYTOPLANKTON CULTURE SYSTEM
PHYTOPLANKTON FEED TANK
FILTERED SEAWATER - 95%
PHYTOPLANKTON FOOD - 5%
SEAWEED GROWTH TANK
SHELLFISH TANK
FINAL EFFLUENT TO COASTAL WATER OUTFALL

FIG. 1 SCHEMATIC FLOW DIAGRAM OF A COMBINED NUTRIENT REMOVAL-MARINE AQUACULTURE SYSTEM

PROCEDURE

The object of the study was to determine the metal content of the secondary effluent, the dilution expected by seawater in the system, and the rate of accumulation of metal by the (cultured) oysters. Oysters have frequently been used as indicators of metal pollution because of their higher sensitivity to elevated levels of many heavy metals[15,16], compared to other species of shellfish[17]. The rate of increase of metal, if present, will indicate the final content of metal expected at the termination of culture, important for public health considerations.

Determination of the content of metal dissolved in the secondary effluent was performed by direct atomic absorption when the metal content was sufficiently high, as often in the case of copper and zinc. Analyses of other elements in effluent and in seawater were done by the APDC-MIBK extraction procedure used by Brewer, Spencer and Smith[18]. With effluent, more MIBK must be used because of its increased solubility, as specified in the EPA standard methods manual[19].

The oysters (Crassostrea virginica) came directly from the shellfish tanks which held 500 individuals. Each tank received daily 595 µg carbon/liter of algae contained in 500 liters of culture[20]. To establish the initial concentration of metal in the oysters, eight individuals were removed at the beginning of the experimental period and analyzed. Later samples of six individuals each were taken from the shellfish tanks after 45 days, 78 days, and 102 days of exposure to the sewage-grown algae.

For wet-ashing, the entire body of an oyster was rinsed with distilled water, weighed and transferred to an aqua-regia-washed 125 ml Erlenmeyer flask. Digestion was performed by adding 10 ml of concentrated nitric acid to the sample, boiling to dryness, and allowing to cool. Then 10 ml of 30% hydrogen peroxide was added and the sample re-dissolved in a 5% nitric acid solution made up of a standard volume for analysis by atomic absorption.

All analyses were done on a Jarrel-Ash Model 800 Atomic Absorption Spectrophotometer (AAS), a dual-beam channel instrument with recorder printout. The most sensitive channel was set to the principal analytical wavelength. The second channel was set to a nearby non-absorbing wavelength, using the "D/A" mode of the instrument[21], to provide correction for matrix effects and background scattering.

Efficiency of the digestion procedure was evaluated by determining recovery and reproducibility. Known portions of metal were added to fifteen five gram amounts of homogenized oyster tissue, with five untreated samples of 5 grams each used for background levels. The metals were added as zinc acetate, copper sulfate, nickel sulfate, cadmium iodide, lead acetate, and potassium chromate. Recoveries of Zn, Cu, Ni, Cd, Pb, and Cr were 97±2%, 95±5%, 98±3%, 98±5%, 96±2%, and 99±3%.

RESULTS

Chemical analysis did not reveal high concentrations of potentially dangerous metals in the effluent obtained from the Otis Air Force Base Sewage Treatment Plant and used in culture (table 1). The metal content generally ran less than considered average for secondary effluent[22], although the copper concentration infrequently rose above .11 µg/ml (ppm) and occasionally above the range expected for average effluent. With the exception of nickel, the concentration of each metal in the effluent generally ran 8 to 150 times the level in the seawater used for dilution. The nickel content of seawater was similar to that of the Otis effluent.

The concentration of metal in the cultured oysters showed a continual decline from the 45th day of culture onwards (figure 2). The apparent increase in concentration during the first 45 days of culture is probably due to our choice of large individuals for the background metal determinations. The later random removal of individuals being monitored for growth indicated that as weight increased, the metal content decreased. Although not premeditated, the mean weight of the samples taken at the termination of culture was similar to the mean of the original samples taken for background (5.68 to 5.18 gms wet meat weight, respectively). A t-test of the difference between mean concentrations at the start of exposure and at the finish revealed no significant differences. With zinc, copper, and lead the total content of metal per individual remained essentially constant, despite changes in weight, while a slight decrease in total metal content occurred with cadmium, chromium, and nickel. Overall, the oysters in the tanks showed slightly over a 50% increase in average weight during the monitoring period, from an initial dry meat weight of .425 gms to a final .676 gms[20].

The original and final concentrations of metal in the meat are also compared with alert levels discussed by the Massachusetts Division of Water Pollution Control[16]. The alert level concept was developed to be used as a guide or indicator of metal pollution in shellfish growing waters and was not intended to reflect the toxicity of metals contained in the shellfish. Only in the case of lead was any indication of contamination given, the baseline value being above the proposed state alert level (table 3), presumably occurring before exposure to sewage-grown algae.

DISCUSSION

Lack of accumulation of metal can be traced to three factors: 1) Dilution of the effluent by seawater, 2) the relatively low metal content of the Otis effluent, and 3) the increased growth of the oysters. Dilution plays by far the most important role of these factors although the mixing of seawater and effluent was determined

Table 1. Constituents of Otis secondary effluent compared with the Washington report "Average" effluent[22]

Material	Concentration (ppm) Secondary effluent Washington Report	Concentration (ppm) Secondary effluent Otis Plant
Organic content (particulate carbon)	25	12-30
(dissolved organic)	10-30	40
Nitrogen (total as N)	20	17.6
Ammonia (as N)	--	15.2
Nitrite (as N)	--	.4
Nitrate (as N)	--	2.0
Phosphorus		
Phosphate phosphorus (as P)	10	8.2
Trace metals		
Chromium	.02-.14	.003-.040
Copper	.07-.14	.025-.300
Lead	.01-.03	.005-.020
Zinc	.20-.44	.035-.095
Cadmium	.01-.03	.0005-.0023
Nickel	.03-.20	.0005-.010

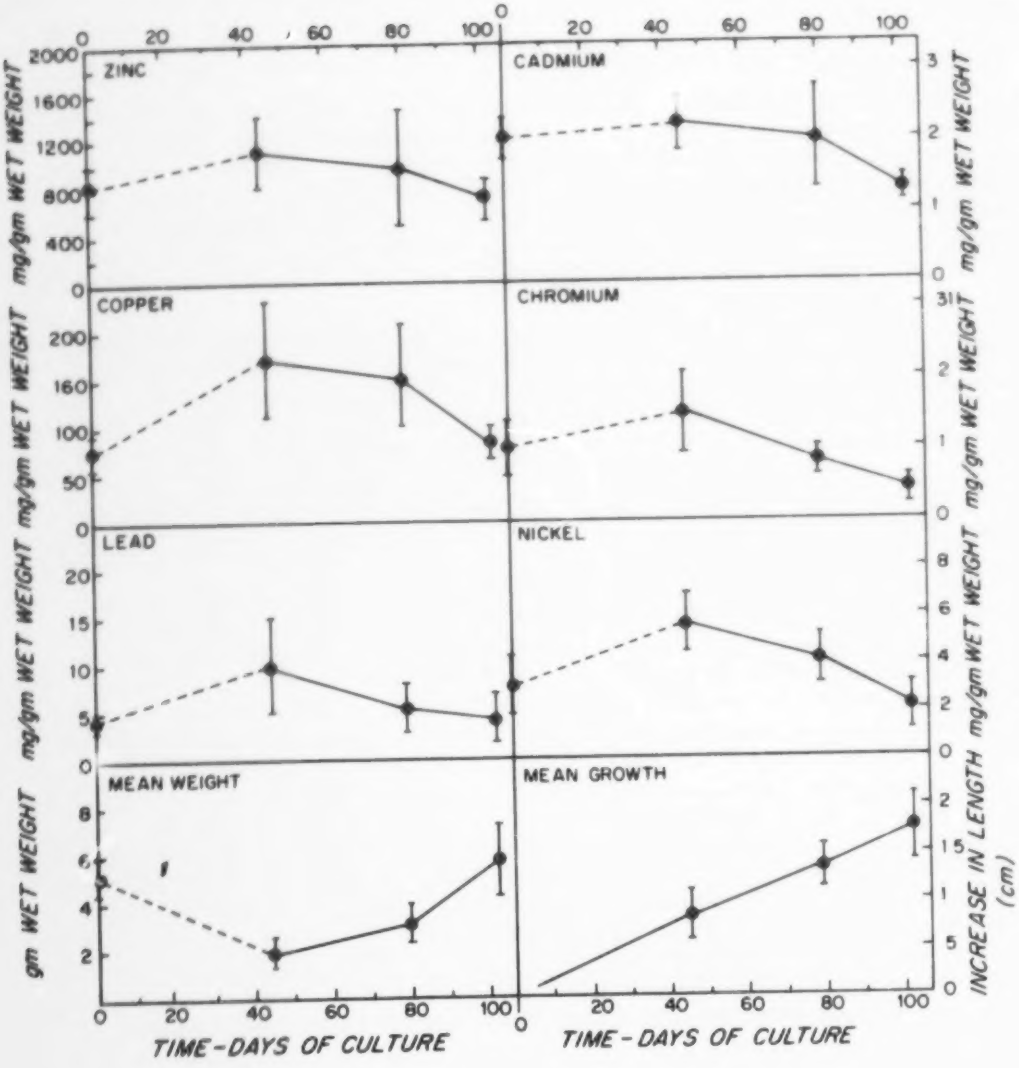

Fig. 2 Metal concentration in tissue of oysters during 102 Days of culture. Mean metal concentration in mg/gm (ppm) wet weight and one standard deviation are included. The mean weight of the individual oysters sampled and their growth, as mean increase of length of shell is also shown

by culture process and not originally intended for lessening metal contamination. The dilution of effluent to seawater for the algae culture mixture was based on earlier experimental results which indicated that yields of algae were roughly proportional to the concentration of sewage effluent in the enriched seawater medium up to concentrations of about 20% sewage and 80% seawater[4]. The dilution of culture mixture with 20 parts seawater as it is delivered to the shellfish tanks was to provide a sufficient volume flow of seawater to maintain a high level of dissolved oxygen to the shellfish[3].

With a very low background metal content, seawater substantially dilutes the original metal levels in the effluent (table 2). For comparison, the amounts of dissolved metal often introduced inadvertently as contaminants in media prepared for algae culture with artificial seawater and nutrients from analytical grade reagents, is equal to or greater than the levels of metals present in the algae cultured prepared from Otis effluent[23].

A total dilution of 1:200 occurs before contact of the effluent with shellfish. This is almost sufficient to reduce the levels of metals in the effluent to the background levels in the seawater (table 2). Even with the element copper, which at times reaches a high concentration in the effluent (300 µg/liter), the maximum level which occurs in water reaching the shellfish is 12 µg/liter, just slightly above the maximum level of copper (9 µg/liter) recorded in the seawater used for culture.

Secondly, the metal content of the Otis effluent, resembling domestic wastewater in character rather than an industrial source, was not particularly elevated. Only copper, of all the elements studied, was as high or higher than the concentrations expected in average effluent as assumed in the Washington report[22] while the chromium and lead content occasionally fell within the average range, zinc, cadmium, and nickel at their highest observed levels were less than the minimum level anticipated in average effluent.

Finally, the increased rate of growth of the oysters probably contributed to the observed decline of metal content in the latter days of culture. For instance, despite the change in lead concentration from 4.2 ± .2 µg/gm wet weight initially to 10.1 ± .5 at 45 days, 5.4 ± 2.2 at 80 days, and 4.1 ± 2.2 at 102 days, the total lead content of the individual oysters, was relatively constant at 21.6 ± 12.0 ug, 20.6 ± 14.8, 19.0 ± 13.0, and 24 ± 12.6, respectively. Copper, and to a lesser extent zinc showed the same constancy of individual metal content despite fluctuations in weight and concentration of metal. In contrast, with cadmium, which previous studies had indicated a very slow loss, if any, within 30 days following exposure to a high concentration[24], some loss did definitely occur after 102 days of culture that was not due to weight change.

Table 2. Concentration of metals in solution in secondary effluent, seawater, and subsequent dilution in algae culture and shellfish tanks. For comparison, the content of metal found in synthetic algae growth media is indicated next to that expected in the culture mixture

Metal concentration µg/liter (ppb)

Element	Otis Effluent	Seawater	Algae Culture Media	Riley's Media	Shellfish Tanks
Zn	35-95	5-12	11-28	150	5-16
Cu	25-300	2-9	7-67	34	2-12
Cd	.5-2.3	.06-.08	.15-.52	nd	.06-.10
Cr	3-40	.3-.5	.9-8.5	12	.3-.9
Pb	5-20	.02-.8	1.0-4.6	31	.07-1.0
Ni	.5-10	1-5	.9-6.0	8	1-5

As concentration of a metal in tissue is a ratio between the total metal content and total weight of the organism, even though a steady increase in total metal content may occur, if the rate of increase in weight is greater, the concentration of metal in the tissue will decrease. For example, the mean total copper content of individual oysters raised from 375, to 304, to 433, and finally to 438 µg on the successive days of sampling while the concentration in µg/gm wet weight (ppm) declined noticeably from the 45 to the 102 day. In the aquaculture system, where growth of the shellfish is accelerated, new tissue is added at a higher rate than that observed normally, serving as a mechanism to dilute the concentration of some metals which may continue to be accumulated at a regular rate regardless of growth.

The 102-day study indicates that oysters can be cultured with the use of secondary effluent as a substitute fertilizer without raising the danger of metal contamination. While other potential contaminants, including pathogens and organic compounds, need further investigation to evaluate the advisability of substituting secondary effluent for artificial media in aquaculture, the initial results suggest that the inherent elevated metal concentrations in domestic wastewater do not appear to pose a threat to shellfish culture if effluent of a principally domestic source is used.

Table 3. Accumulation of metals in oysters of pilot tertiary treatment-aquaculture system fed sewage-grown algae for 102 days. Mean metal concentration and one standard deviation are indicated

	Metal content (ppm wet weight)		
Element	Initial	Final	State Alert Level[16]
Zn	818 ± 446	743 ± 182	2000
Cu	72 ± 18	81 ± 18	175
Cd	2.00 ± .30	1.30 ± .26	3.5
Cr	.10 ± .04	.04 ± .02	2.0
Pb	4.2 ± .2	3.6 ± 2.5	2.0
Ni	.29 ± .13	.21 ± .10	Unspecified

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PERMISSIBLE LEVELS OF HEAVY METALS IN SECONDARY EFFLUENT FOR USE IN A COMBINED SEWAGE TREATMENT-MARINE AQUACULTURE SYSTEM II. DEVELOPMENT OF GUIDELINES BY METHOD OF ADDITIONS*

by

W. B. Kerfoot and G. A. Redmann

Shellfish can be cultured on algae raised with secondarily-treated wastewater substituted for artificial nutrients without any apparent accumulation of toxic metals in their tissues[1]. The effluent used was found to be relatively low in background concentrations of metals, similar to principally domestic sources using anaerobic treatment procedures. In many of our industrialized cities, however, the effluent from a sewage treatment plant contains an array of metals at substantially higher levels than that received from solely domestic sources[2,3]. Rather than test each source individually, it would be worthwhile to develop guidelines which serve to define suitable sources of effluent for aquacultural purposes. Within this paper we attempt to determine the concentrations of six metals, zinc (Zn), copper (Cu), lead (Pb), cadmium (Cd), chromium (Cr), and nickel (Ni) that are permissible in a combined sewage treatment-marine aquaculture system.

TERTIARY TREATMENT-MARINE AQUACULTURE

Nutrient-laden secondary effluent can become a nuisance when discharged into coastal waters, stimulating algae blooms with foul odor and contaminating shellfish beds. On the other hand, careful control of the quality of the effluent to assure a pathogen-free high nutrient source will provide a potentially valuable resource in the face of diminishing supplies of food and artificial fertilizer. Secondarily treated wastewater, diluted with seawater, makes an excellent medium for growing marine phytoplankton. The phytoplankton is, in turn, removed from the water by filter-feeding shellfish, converting the organically-fixed nutrients into valuable protein.

During the past three years, a prototype system of tertiary treatment-marine aquaculture has been in operation

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at Woods Hole Oceanographic Institution. Various designs have been engineered to culture marine phytoplankton and subsequantly raise shellfish, with any nutrients regenerated by faecal material of the shellfish being removed by the growth of macroscopic algae in tanks before discharge to coastal waters[4,5,6].

A more detailed explanation of the system employed during the summer of 1972, upon which these experiments are based, is explained in the previous paper (Kerfoot and Jacobs)[1]. Effluent was added to filtered seawater at a ratio of 1:4. Phytoplankton cultured in this mixture together with diluting seawater was then introduced into tanks of shellfish at a concentration of one part culture to 19 parts 100 u-filtered seawater. At this dilution, the carbon content of the seawater, an indicator of the food value to the shellfish, is raised from a normal level of 100 ug carbon/liter to roughly 600 ug carbon/liter. More than 80% of the phytoplankton fed to the shellfish originates from nutrients provided by the effluent, and about 1% of the water reaching the shellfish is derived from the secondary effluent.

PATHWAYS OF CONTAMINATION AND TOXICITY

When metals are introduced with the effluent, the algae may accumulate metallic ions directly from solution by absorption (here indistinguishable from adsorption). Then the algae mixture flows into the herbivore tanks, where the shellfish may directly absorb ions from the considerably diluted culture solution or through ingestion of contaminated phytoplankton and detritus (figure 1). In this study the algae cultured was *Phaeodactylum tricornutum*, a pennate diatom commonly occurring during normal operation of the aquaculture system. The American oyster (*Crassostrea virginica*) was chosen as the shellfish species because of its potential economic value as a luxury food and the need to assess its sensitivity to metals which may be added in the effluent.

The sensitivity of *Phaeodactylum tricornutum* to change in metallic ion concentrations is well recognized. Previously Hayward has shown that *P. tricornutum* initially absorbs zinc rapidly, but the concentration in cells decreases as their numbers increase[7]. Riley and Roth found that *P. tricornutum* under similar conditions accumulated less than half the amount of metals that Hayward found[8]. Nuzzi reported that mercury levels as low as .06 ug/liter (ppb) were inhibitory to *P. tricornutum*, and that morphological abnormalities increased with mercury concentration,

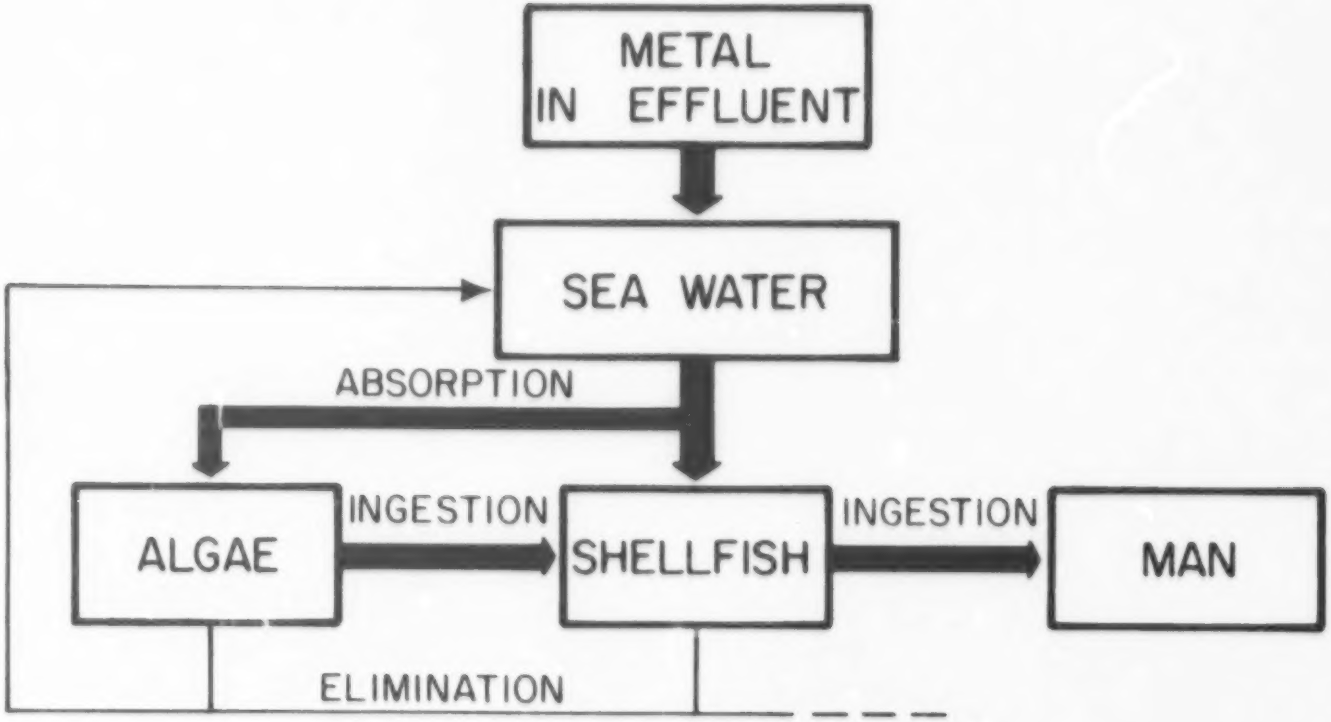
METAL
IN EFFLUENT
SEA WATER
ABSORPTION
ALGAE
INGESTION
SHELLFISH
INGESTION
MAN
ELIMINATION

Figure 1. Pathways of contamination in the treatment-aquaculture system.

the normally biradiate cells becoming highly vacuolated and assuming an ovoid state[9].

Hannon and Patouillet noted that growth inhibition by a toxicant varies inversely with the concentration of nutrients available. They found that the exponential growth of P. tricornutum was not affected by exposure to 0.1 ppm lead or copper. Exposure to 1.0 lead had no effect, while 1.0 ppm copper inhibited the growth rate to less than half that of controls[10].

The ability of the American oyster to accumulate trace metals from solution is also well-documented. Roosenberg founr that greening of oysters could be traced to excessive uptake of copper due to thermal effluent from power plant activity[11]. Pringle et. al. surveyed the average trace metal levels in shellfish from the Atlantic and Pacific coasts and also carried out studies on metal uptake and concentration in a simulated natural system[12]. They reported that lead was absorbed by C. virginica in direct proportion to concentration in seawater, accumulating four migrograms of Pb per gram wet meat per day when exposed continuously to 0.2 ppm in seawater. Average metal backgrounds in Atlantic oysters were: zinc, 1428 ppm; copper, 92 ppm; lead, 0.47 ppm; nickel, 0.19 ppm; chromium, 0.40 ppm; and cadmium, 3.10 ppm. Kerfoot and Jacobs found linear cadmium uptake by C. virginica from seawater in a deliberately contaminated sewage treatment-aquaculture system[13]. The observed rate of uptake of cadmium (in ug/gm/day) was 17.8 times the concentration of cadmium in solution (in ppm-ug/ml). With effluent as the source of cadmium, the principal means of uptake was through the ingested algae.

MATERIALS AND METHODS

Scale models of a sewage treatment-aquaculture system were constructed in the seawater laboratory at Woods Hole Oceanographic Institution (figure 2). Two-liter erlenmyer flasks were filled daily with a 4:1 mixture of one micron filtered seawater and filtered (.45 u) secondary effluent from the Otis Air Force Base sewage treatment plant. Effluent containing 10 ppm, 3 ppm, .3 ppm, and .03 ppm equal concentrations of Zn, Cu, Pb, Ni, Cd, Cr was added to the flasks with one flask receiving unsupplemented effluent as a control. The effluent-seawater mixture, as media, was dripped continually at 1.5 ml per minute or 2 liters per day into 4-liter flasks containing cultures of the diatom species. Water containing the cultured diatoms flowed at the same rate from the culture flasks into trays holding

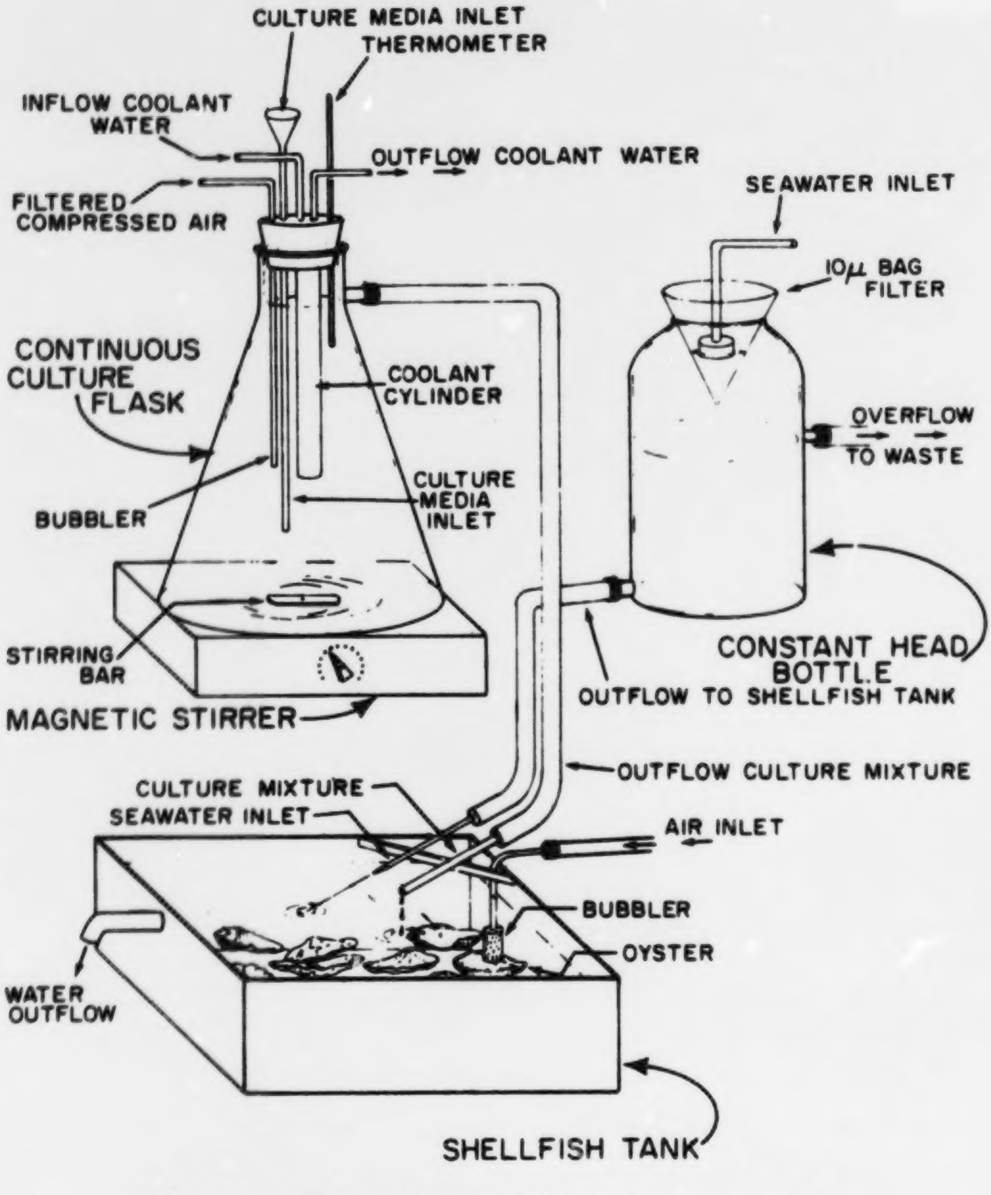
CULTURE MEDIA INLET
THERMOMETER
INFLOW COOLANT WATER
OUTFLOW COOLANT WATER
FILTERED COMPRESSED AIR
SEAWATER INLET
10μ BAG FILTER
CONTINUOUS CULTURE FLASK
COOLANT CYLINDER
OVERFLOW TO WASTE
CULTURE MEDIA INLET
BUBBLER
STIRRING BAR
CONSTANT HEAD BOTTLE
OUTFLOW TO SHELLFISH TANK
MAGNETIC STIRRER
OUTFLOW CULTURE MIXTURE
CULTURE MIXTURE
SEAWATER INLET
AIR INLET
BUBBLER
OYSTER
WATER OUTFLOW
SHELLFISH TANK

Figure 2. Scale model treatment-aquaculture system.

oysters in two liters of water, where it was mixed with 25 ml per minute of filtered seawater supplied from a constant head tank.

The culture flasks were illuminated by a bank of fluorescent bulbs providing approximately 500 ft-candles, stirred by magnetic stirring bars, and maintained at 20° ±2° C by seawater flowing through glass tubing inserted into the culture mixture. The culture also received air, cleaned by a water trap and activated carbon, bubbled continually through the mixture from an inserted pipette. Four 20 liter plastic carboys were filled with .45 u-filtered secondary effluent. The stock solutions were made up to 10.0, 3.0, 0.3, and 0.03 ppm (mg/l) with zinc from zinc acetate ($Zn[C_2H_3O_2]\cdot 2H_2O$), copper from copper sulfate ($CuSO_4\cdot 5H_2O$), cadmium from cadmium iodide (CdI_2), lead from lead acetate ($Pb[C_2H_3O_2]\cdot 3H_2O$), chromium from potassium chromate (K_2CrO_4), and nickel from nickel sulfate ($NiSO_4\cdot 6H_2O$). Each day of the study, 400 ml of effluent or metal-enriched effluent from the four carboys was mixed with 1600 ml of one micron-filtered seawater in the 2 liter media flasks which supplied each algae culture flask.

Fluorimeter readings were taken daily on 10 ml samples of the algae cultures. Weekly samples were taken of the oysters and frozen after being placed in clean seawater to void their intestinal tracts. Samples of the seawater and effluent were removed periodically during the study for analysis of metal content. At the termination of the experiment, two liter samples of the algae were removed from the culture, centrifuged, and dried on nuclepore filters for metal analysis.

The procedure employed for wet-ashing was the same as that reported by Kerfoot and Jacobs[1,13]. The entire body of an oyster was removed from the shell, rinsed with distilled water, weighed, and transferred to aqua-regia washed 125 ml erlenmyer flasks. Digestion was performed by adding 10 ml of concentrated nitric acid to the sample. The sample was allowed to stand in acid for 12 hours at room temperature, heated gently close to dryness, and allowed to cool. Then 10 ml of 30% hydrogen peroxide was added and the sample heated to dryness. After cooling, the sample was redissolved in a 5% nitric acid solution, made up of a standard volume for analysis, and aspirated directly into the atomic absorption spectrophotometer. All analyses were done on a Jarrell-Ash Model 800 Atomic Absorption Spectrophotometer, using a nearby non-absorbing line for background absorption.

Phytoplankton samples on the filters were added to 10 ml of 50% nitric acid in individual 20 ml test tubes. After 48 hours digestion, the solution was filtered through 3 u nuclepore filters into 20 ml centrifuge tubes for direct aspiration into the atomic absorption spectrophotometer.

Metal analysis of the seawater and effluent was performed using the techniques described previously by Kerfoot and Jacobs[1]. The samples were filtered and analyzed directly by atomic absorption or were extracted and analyzed. For solvent extraction, four 500 ml samples of seawater or effluent were taken, adjusted to a pH of 2.0, and then extracted twice with MIBK-APDC. Graded amounts of the individual metal were added at levels 1, 2, and 3 times higher than anticipated in solution. The concentration in solution could then be calculated following the method of additions reported previously by Brewer, Spencer, and Smith[14]. The concentration of background metals in the effluent used for stock solution was .080, .055, .005, .002, .010, and .005 ppm for Zn, Cu, Pb, Cd, Ni, and Cr. Metal content of the seawater and effluent supplied to the cultures was reported earlier in the preceding article[1].

The culture system was continued for 28 days, during four of which the effluent media mixture did not flow, for varying reasons. During this time the algal populations fluctuated somewhat. To maintain a suitable level, it was sometimes necessary to supplement the experimental cultures from stock cultures.

To further define toxic effects of specific metals on the phytoplankton, four separate experiments were devised with batch cultures of P. tricornutum.

First of all, eight 250 ml flasks were prepared by the addition of 80 mls filtered seawater and the effluent stock solutions, two flasks at each multiple metal concentration. These were autoclaved and aseptically inoculated with 6×10^5 cells for each flask from a parent culture in exponential growth. All batch cultures were maintained in a growth chamber at 18° C, 450 ft-candles, and a light:dark regime of 13:11. Samples of 1 ml were taken every other day for 10 days, preserved in Lugel's solution and counted in either a Speirs-Levy or Palmer-Mahoney counting chamber.

A second series consisting of twenty-four 125 ml flasks with 50 ml of effluent-seawater media was prepared with 6 different 5-metal mixtures at 3 concentrations: 5 ppm, .5 ppm, and .05 ppm metals in the effluent. Each flask was inoculated with 4.4×10^6 cells, producing 8.8×10^4 cells/ml. The five-metal solutions were added to the cultures three days after inoculation and samples counted at 5, 8, and 17 days after inoculation.

A third series to determine relative toxicity of each metal consisted of eight 250 ml flasks, using 100 ml of the 4:1 seawater/effluent media, with six receiving a single metal at a concentration of .5 ppm in the effluent and two controls. These were inoculated with 4.4 x 10^5 cells. Samples were taken 4, 6, and 7 days after inoculation.

A fourth experiment tested the relationship between cell density and metal inhibition. Six 250 ml flasks holding effluent/seawater media and the 3 ppm mixed metal solution added to yield a solution concentration of .6 ppm mixed metals. Then .1, 1.0, and 10.0 ml of inoculum were added to each pair of flasks, experimental and control. The cells were sampled and counted 1, 3, and 4 days after inoculation.

RESULTS

PHYTOPLANKTON TOXICITY

During the course of the study, the levels of algae varied greatly (figure 3) in the scale-model treatment-aquaculture systems. The concentration of metals in the effluent media had little influence on the algae abundance. The mean density of *P. tricornutum* in fluorescence units (fu) was 1060, 1157, 1090, 1360, and 1178 fu, respectively, for 10.0, 3.0, 0.3, 0.03 and control. Reduction in the *P. tricornutum* cultures occurred primarily from predation by flagellates which passed through the 1.0 micron filters and grazed down the diatoms. However, the cultures supplied with higher concentrations of metals in the media exhibited a more rapid decrease in cell concentration once a drop began. Also, the cultures receiving 10.0 and 3.0 ppm mixed metals in their effluent would not recover without supplemental additions of culture.

With a series of experiments, the relative contribution of each metal to toxicity was isolated (figure 4). When *P. tricornutum* was cultured in a mixture of all six species of metals, growth was inhibited when concentrations exceeded 0.3 ppm. In all series with five-metal combinations, all showed reductions in algae growth in cultures of low density at .05 ppm and above concentration, except when copper was removed from the combination (figure 4, middle). The remaining metals (Zn, Pb, Cd, Cr, and Ni) did not cause a drop in algae growth until .5 ppm concentration and above in

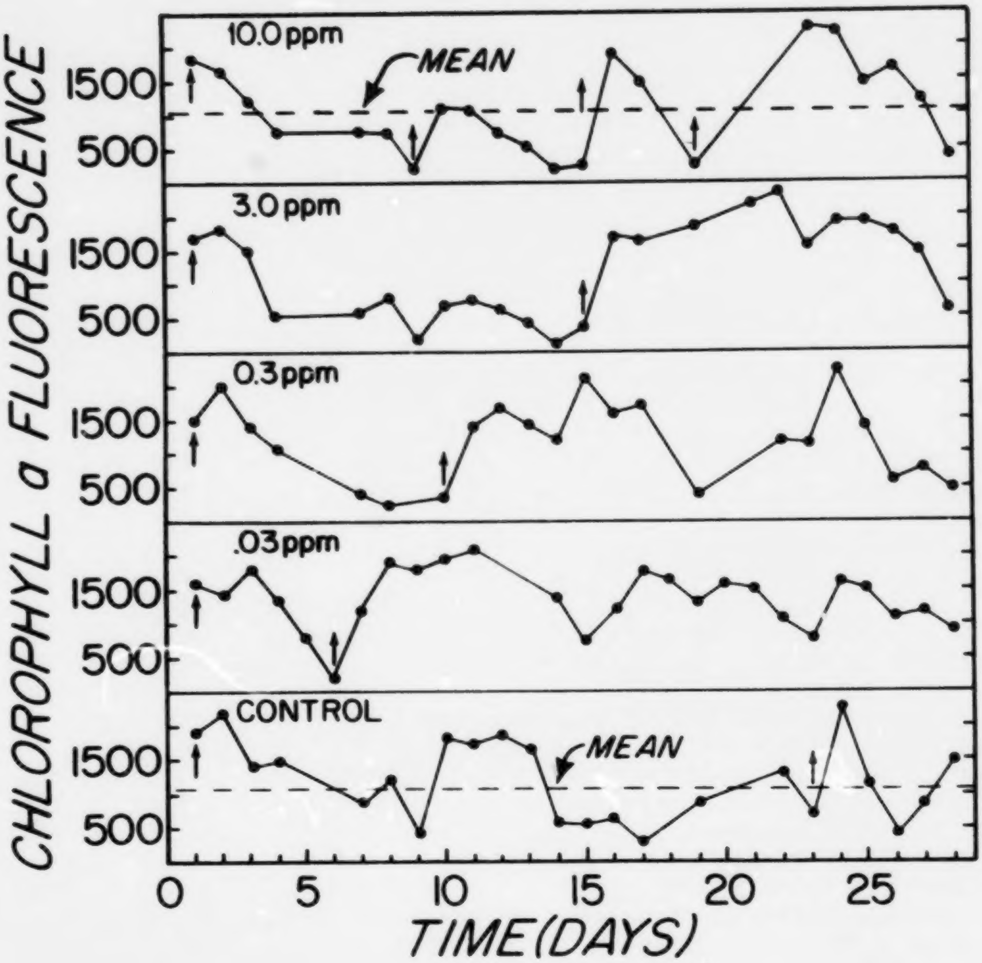

Figure 3. Concentration of phytoplankton (in fluorescence units) in culture flasks during operation of experimental systems. The concentration of metals supplemented in the effluent indicated for each culture. Arrows mark additions of algae from stock culture.

the effluent. The metals having the principal toxic effect in the five-metal combinations (minus copper) were Cd and, to a lesser extent, Ni. When metals were added individually at .5 ppm concentration in the effluent, copper (Cu), cadmium (Cd) and nickel (Ni) had a pronounced depressing effect on algae growth.

From the toxicity series, levels of metal above .3, .5, .5, .5, 1.0, and 1.0 ppm, respectively, for Cu, Cd, Ni, Cr, Pb, and Zn may inhibit algal growth if present in the effluent and would appear undesirable. The limit of toxicity found for copper agrees with the report of Mandelli (1969) that the growth of four different marine diatoms was inhibited by Cu levels in seawater greater than .05 ppm, equivalent to .25 ppm Cu in effluent before dilution[15].

Other effects observed on the diatoms were certain morphological variations. Nuzzi previously described abnormalities of P. tricornutum induced by mercury[9]. At 2.0 ppm in the culture media, corresponding to the use of 10.0 ppm mixed metals in the effluent, misshapen chromatophores and double nuclei occassionally occurred, as well as "fat" or foreshortened cells of this normally biradiate pennate diatom. Somewhat shrunken cells and paired nuclei were found down to the .3 multiple metal level. A bifurcation at one end of the cell or "fishtail" was often found in all the cultures, including the controls fed with effluent unsupplemented with metals.

The influence of cell density on the expression of toxicity was also apparent (figure 5). Initial cell concentrations ranging over three orders of magnitude were added to media prepared with 3.0 ppm each of the six metals in the effluent. As the cell concentration rose, the toxicity decreased. This supports the observation that the high density of cells being cultured in the aquaculture system suppresses toxicity until a drop in cell number occurs. The drop is then accentuated by elevated levels of metal introduced in the effluent. Concentrations of metals exceeding the toxic levels suggested have an important effect in decreasing the stability of the culture and its ability to recover from any slumps in production.

OYSTER TOXICITY

Few oysters died during the operation of the scale model treatment-aquaculture systems. In no case could the isolated deaths be attributed to metal concentrations. This is likely due to the high dilution (100x) of effluent before exposure to the oysters.

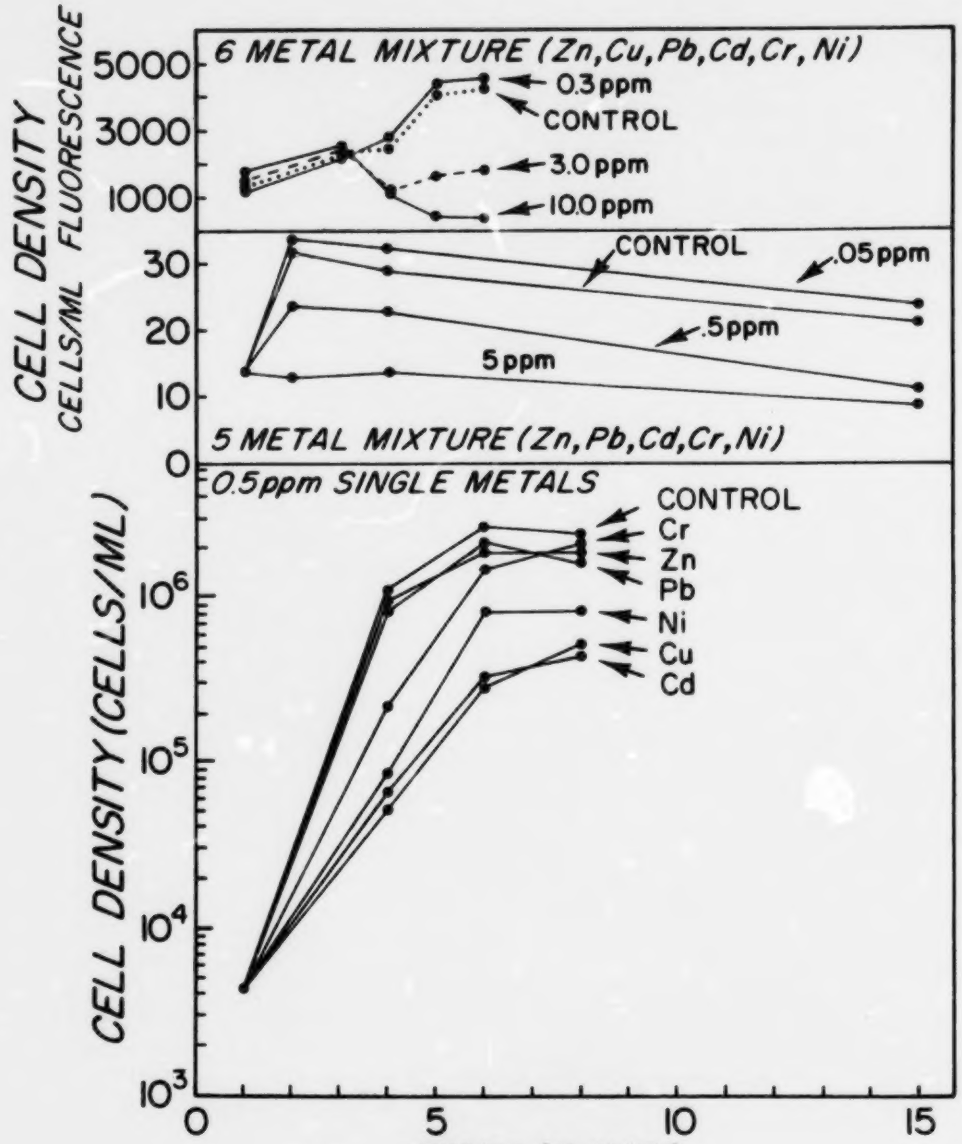

Figure 4. Effect of metals on diatom growth.

ABSORPTION BY PHYTOPLANKTON

All of the elements examined were acumulated by the phytoplankton (Table 1). At the highest concentration of metals added in the effluent (10.0 ppm), equivalent to 2,000 ug/liter since it is diluted five-fold with seawater, the respective percentages of metal removed from solution by the algae (.1 gm/liter dry weight) were 59.0, 16.9, 14.9, 9.6, 4.6, and 2.5% for Pb, Zn, Cu, Cd, Ni, and Cr. Five of the metals remained relatively stable in their order of magnitude of accumulation, Zn>Cu>Cd>Ni>Cr. However, lead (Pb) showed a rapid increase in percentage absorption, ranging from undetectable at .03 ppm concentration to 59% of the total lead added in solution from the effluent concentration of 10.0 ppm.

Of interest to those who may feel that the use of effluent as a media will cause high background levels of metal in the cultured algae, the levels of metal in the controls raised on unsupplemented effluent were generally lower than those found by Riley and Roth for P. tricornutum cultured in artificial media containing low levels of trace elements[8]. Riley and Roth found background levels of 325, 110, 46.3, 6.2, and 4.4 ppm in dry plankton for Zn, Cu, Pb, Ni, and Cr. We found 120.8, 60.4, 14.6, 12.5, and 4.03 ppm, respectively, for the same elements. While it should be kept in mind that Riley and Roth used a dc arc spectrographic technique for analysis, thereby likely analyzing some additional metal in the silicaceous skeleton of the diatom, the levels are quite comparable.

ACCUMULATION BY OYSTERS

An increase in the level of metals in the seawater/effluent media, in general, caused a progressive rise in the content of metal in the tissue of the oysters being cultured (figure 6). The points represent the mean metal analyzed from three or four oysters. The clearest pattern of accumulation occurred in the elements of lesser affinity for the oyster tissue, Cr, Ni, and Cd. The concentration of lead showed a more abrupt rise in level than the other metals. Cadmium was the only element to show detectable accumulation at .03 ppm, but also appeared to saturate at the highest level (10.0 ppm). Some accumulation of copper occurred, but no definite uptake of zinc could be discerned. With zinc and copper, to some extent, the amount absorbed, even though it may have been large in quantity compared to the other elements, was obscured by the high background metal content of the tissue.

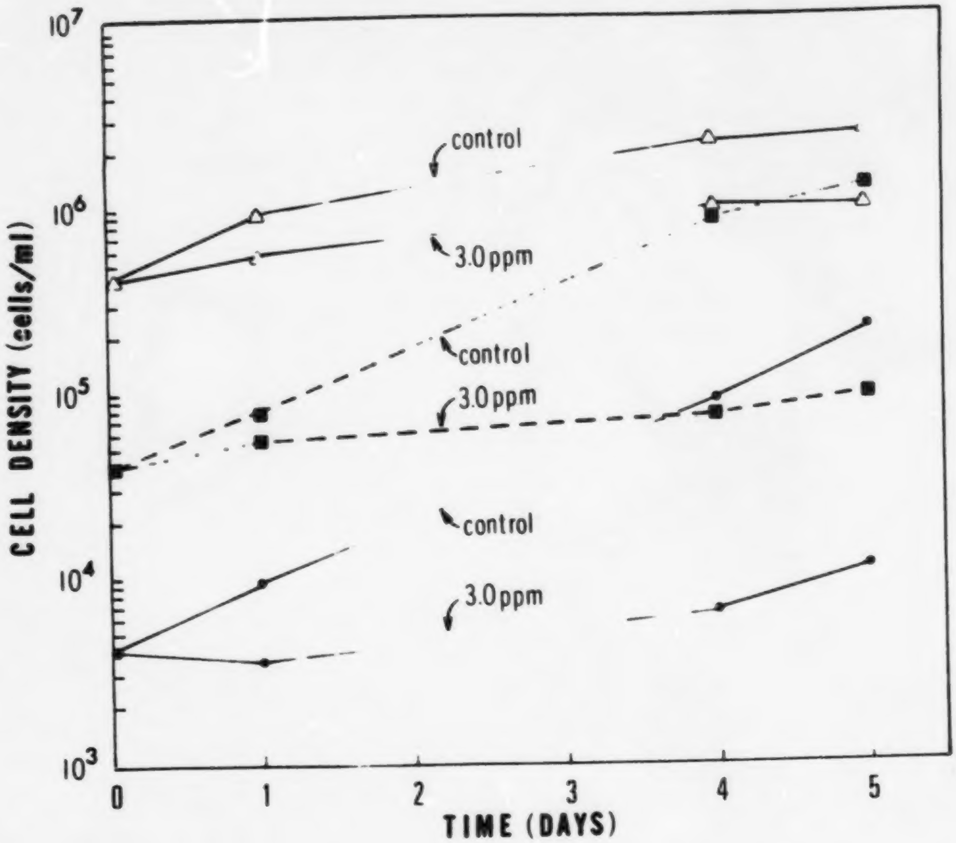

Figure 5. Influence of cell density on the expression of toxicity.

Table 1. Metal content of phytoplankton (ug/gm dry weight).

Metal Added to Effluent	Zn	Cu	Pb	Cd	Cr	Ni
Control	121±8	60±4	14.6±.7	3.4±.6	4.0±1.4	12.5±9.0
.03	113±3	63±8	11.2±1.8	14.2±1.1	9.7±1.2	2.6±.8
.3	396±13	159±13	57±21	124±3	48±2	82±7
3.0	592±18	634±79	551±127	574±104	67±15	161±19
10.0	3510±290	3050±450	11,800±1366	1930±260	498±59	932±53
Background (Riley and Roth)[8]	325	110	46.3	n.d.	4.4	6.2

n.d. = not determined

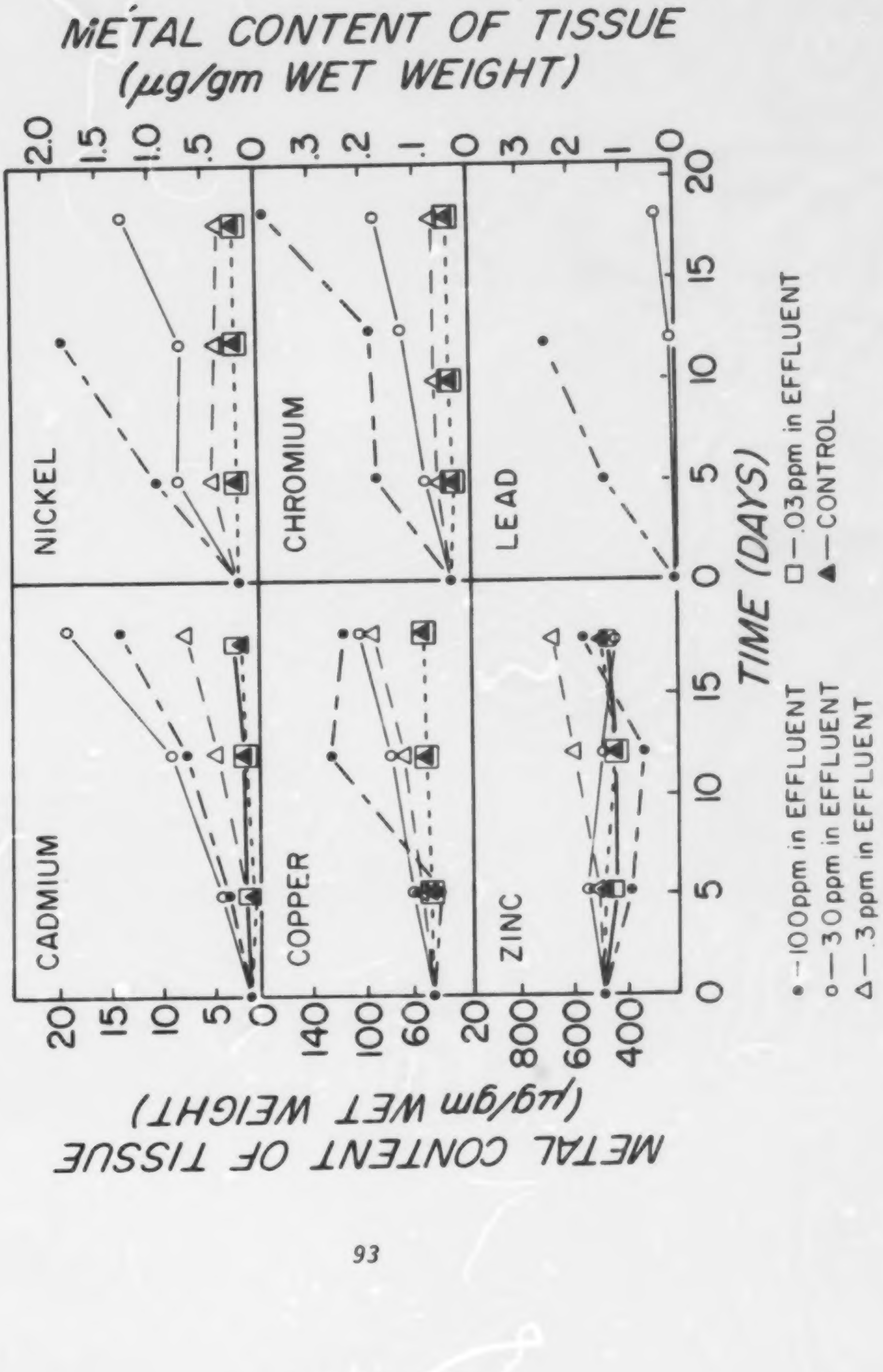

Figure [illegible]. Accumulation of metals by oysters.

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When the rate of increase was plotted versus the concentration of metal added in the effluent, a disposition of uptake similar to that encountered with the phytoplankton occurred (figure 7). Each point represents the mean rate of uptake from each series in figure 6. The order of affinity for the four metals with roughly parallel slopes was Cu>Cd>Ni>Cr. The rate of uptake of lead (Pb) was over twice the rate of the other metallic species and undetectable below 3.0 ppm in the effluent. The abruptness of accumulation was similar to that observed with the phytoplankton.

Although Cu, Cd, Ni, and Cr exhibited similar rates of accumulation, the fitted lines all had a slope of less than 1.0. This indicates that the magnitude of metal uptake decreases with increasing concentration. A previous detailed study of cadmium uptake in the treatment-aquaculture system indicated a relatively fixed ratio of uptake by oysters over a wide range of metal concentration in both effluent and seawater as sources. If the combination of metals acted synergistically, the effect was to impede uptake of metallic ions at the higher concentrations.

DEFINING A LIMIT

Ideally, the permissible level of a metal should be one which allows the organisms of the culture system to be free from acute or chronic toxicity and the food products to be entirely safe for human consumption. With this in mind a series of limits were prescribed based on phytoplankton toxicity, shellfish toxicity, acute toxicity to human consumers, chronic toxicity to human consumers, and finally, pollution alert levels. The results are presented in Table 2.

First of all, the levels of concentrations of metals which bring about inhibition of growth of Phaeodactylum tricornutum are listed in the first row. These are based on the continuous culture and batch culture tests performed on 6-metal, 5-metal, and single-metal additions in the effluent as reported in this paper.

Secondly, since no toxic effects of metal additions were observable with the oysters, the permissible levels were set at 10 ppm. Further experiments conducted for periods of time longer than 28 days may well show chronic toxic effect as metals accumulate in the tissues of the oysters. However, no acute effects were noticeable.

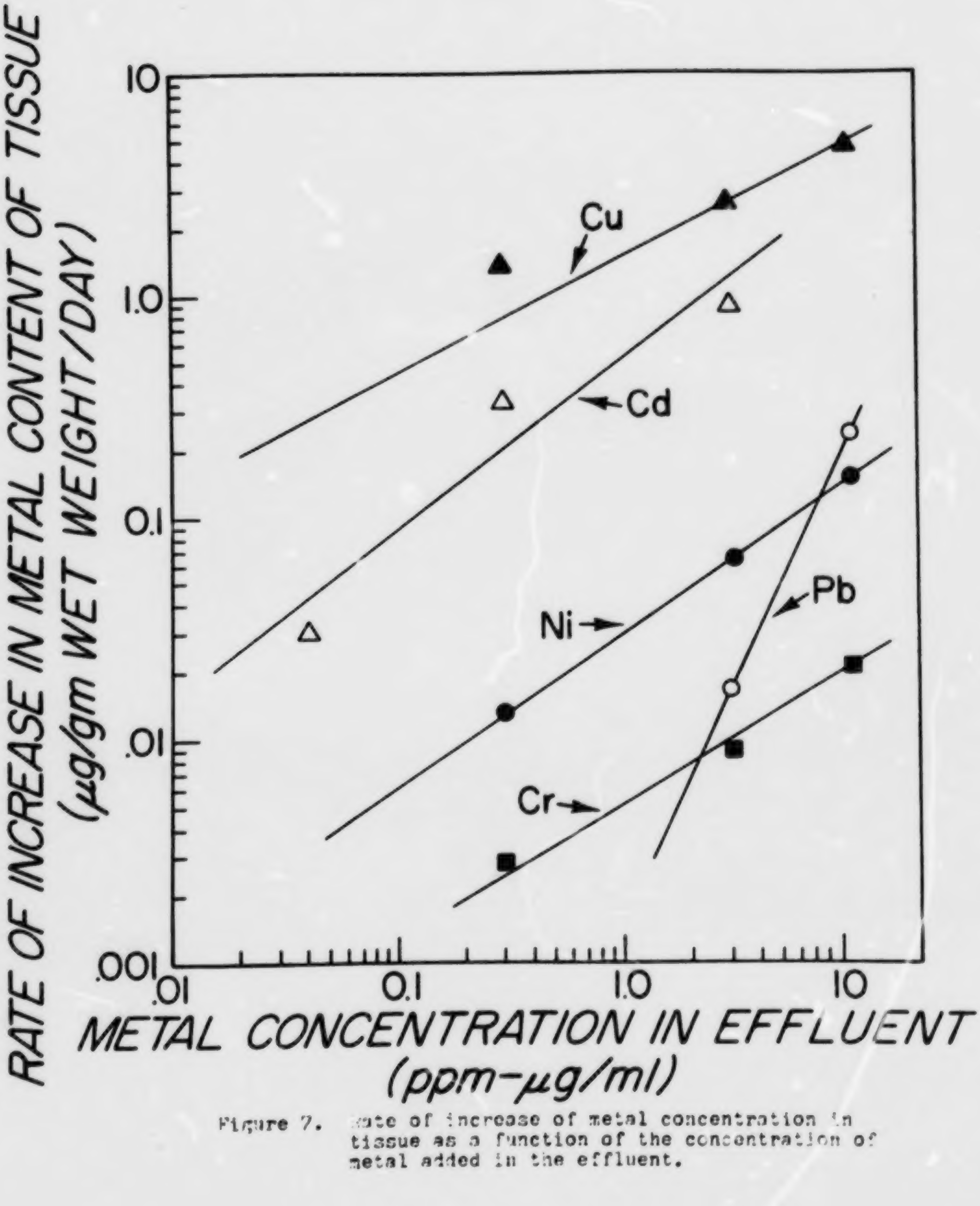

Figure 7. Rate of increase of metal concentration in tissue as a function of the concentration of metal added in the effluent.

Table 3. Concentrations of metal permissible in solution in secondary effluent for use in a combined tertiary treatment-marine aquaculture system using a 1:4 dilution of effluent for algae media and a 1:19 dilution of algae culture for raising oysters.

	Concentration of Metal in Effluent (ppm)					
Based on:	Cu	Cd	Ni	Cr	Pb	Zn
Algae Acute Toxicity	.3	.5	.5	.5	1.0	1.0
Shellfish Acute Toxicity	10	10	10	10	10	10
Human Acute Toxicity	.2	.080	N.S.	>10.0	N.S.	N.S.
Human Chronic Toxicity	N.S.	.010	N.S.	N.S.	6.0	N.S.
Pollution Alert Level	.36	.012	.05	3.0	2.4	N.S.
Suggested Guideline	.2	.010	.05	.5	1.0	1.0

N.S. = Not Specified

A number of the metals studied are known to have toxic effects when present in food in high amounts. Copper has been reported to cause bitterness and gastroenteritis when present in high levels in oysters[11,16]. The excessive copper also imparts a greenish tinge to the oyster meat. As a result, Roosenburg has suggested a 100 ppm level of copper in tissue be set for adult oysters for human consumption. Browning has reported that one to two grains of copper as sulfate will produce severe abdominal pain, vomiting, and diarrhea when ingested[17]. One grain is equivalent to a dose of 6.6 mg. For a meal of 40 gm of oyster meat, equivalent to eight 5-gram oysters, this would represent a concentration of 180 ppm in their tissue. The acute toxicity level is computed in Table 2 on the basis of a 100 ppm limit on concentration, which would necessitate a daily increase of .5 ug.

To reach a concentration of 100 ppm in a 5 gm oyster after 3 years of culture (roughly 1000 days - the average time needed) the oyster would have to accumulate 500 ug of copper, requiring an uptake rate of .5 ug/day. From figure 7, this would occur with a .2 ppm concentration of copper in the effluent used for culture.

Schwarze and Alsberg found that the maximum amount of cadmium in food that could be tolerated without producing emesis was roughly 400 ppm[18]. However, reported instances of poisoning since then have lowered the emetic threshold concentration to 13 ug/gm cadmium[19,20]. This level requires an uptake rate of .065 ug cadmium/day by the cultured oysters. A concentration of .080 ppm in the effluent would be necessary to reach that level after 3 years (1000 days) culture.

No acute toxicity from ingestion of nickel has been reported for the levels likely to be encountered in shellfish culture. Systemic poisoning from nickel salts is almost unknown, although dermatitis has occassionally been noted[17]. Nickel carbonyl poisoning has been well-documented but is unlikely to occur in the effluent used for aquaculture purposes.

By oral administration, elevated levels of chromate (as potassium) cause stunting of growth. Gross and Heller reported that the maximum amount that can be added to feed without causing toxic effects was 1/8 of 1 percent, a concentration of 1250 ppm Cr[21]. To reach this level would require a rate of uptake in excess of 6.2 ug/day, requiring concentrations in the effluent greater than 10.0 ppm, unlikely to occur.

There is little point in computing limits based on acute toxicity for lead or zinc, the two remaining metals. Very little ingested lead or zinc is absorbed in the intestines. Intakes of zinc in the form of chloride or carbonate equal to 2,500 ppm of the diet show little effect on animals[17].

Of the six metals, cadmium and lead are known to cause chronic toxicity from long-term accumulation from ingested food in humans. We are not aware, at present, of any reported cases of poisoning which can be traced to concentrations of either of these elements in oyster meat. However, the critical levels in meat are known or have been estimated for each of the metals and can be calculated.

Kerfoot and Jacobs, using a model similar to that employed by Kjellstrom to set cadmium standards in air, computed that concentrations of cadmium in oyster meat above 3.0 ppm would lead to a critical level in the kidney cortex if 40 gm of oyster meat were ingested daily for 50 years[13]. This would require a daily rate of uptake of .015 ppm for the 1000 day culture period. A concentration of .010 ppm in the effluent would be necessary to reach this level.

According to Kehoe, any level of lead greater than a total of .6 mg/day is potentially dangerous[22]. Using the generous estimate of 40 gm of oyster meat per day as the diet, a concentration of 15.0 ppm would be undesirable. This requires a rate of uptake of .75 ug/day for the 1000 day culture period. A concentration of 6.0 ppm in the secondary effluent would bring about this eventual level.

Finally, a series of permissible levels were computed on the basis of alert levels at one time considered for indicators for pollution for the State of Massachusetts[23]. The alert levels for Zn, Cu, Cd, Cr, and Pb were, respectively, 2000, 175, 3.5, 2.0, and 2.0 ppm wet meat weight. Nickel was not specified. The alert levels represent the upper value three standard deviations from the mean level found in unpolluted areas. This was not meant to indicate a significance of public health but to serve only as signal of an unnatural source. Since the standard deviation of nickel content in oysters is about ±.13, the alert level based on a .29 ppm natural concentration in the tissue would be about .70 ppm.

Permissible levels, based on the alert levels, were calculated in the final row of Table 2. Since zinc was not accumulated significantly during the study, no limit was calculated for it. As before, the culture period was assumed to be 1000 days, resulting in a 5 gram oyster.

The final permissible level suggested as a guideline was taken to be the lowest value occurring in the column for each metal. Effluent containing a concentration of metal in solution above or at this level is not recommended for aquacultural purposes. In three cases, Cr, Pb, and Zn, the suggested level represented the concentration toxic to algae. For copper, the level was based on acute toxicity to humans. With cadmium, it reflected chronic toxicity of the metal in humans. Oddly, nickel was the only element whose recommended concentration in the effluent was based on alert levels, representing a level three standard deviations above that normally found.

Even though the levels of metal in the effluent for use in the aquaculture system were calculated against standards suggested for food regulations, many questions are left unanswered as to whether consumption of the shellfish is completely safe from a public health point of view, considering only metals. Total metal content may be misleading since the structure of the compound is not elucidated. Organometallic species of metals may be present in effluent due to industrial discharge or possible reactions encountered during the sewage treatment process. These compounds, if present, may have toxic effects not commonly associated with their inorganic counterparts. Also, the possible carcinogenic, mutagenic, and teratogenic effects were not considered here and should be investigated.

The many difficulties which beset the definition and validity of standards should not delay progressing towards guidelines, despite their tentative nature. Much will be gained by establishing the proper limits for metals in effluents to be used for aquaculture, although the definition of precise limits to cover all possible toxic effects may take a long time to develop, particularly since many oysters sold commercially have been unintentionally exposed to discharge of municipal effluents, retaining elevated levels of metals despite depuration for possible pathogenic organisms.

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CALCULATED YIELD OF SEWAGE LAGOON BIOMASS, A PLAN FOR PRODUCTION, AND SOME OF THE PROBLEMS INHERENT IN USING BIOMASS OR LAGOON WATER FOR PRODUCTION OF FOOD AND FIBER

BY

KARL SCHURR*,** and J. M. GOLOMBEK***

INTRODUCTION

Lagoon treatment of sewage is rapidly expanding both in the United States and throughout the world. It is certainly opportune and appropriate that we should be meeting to exchange information about our difficulties and successes in the application of research to sewage lagoon operation and technology. The focus of this paper will be on the Deshler, Ohio terminal sewage lagoon system. This is an aerobic lagoon which is 5.4 hectares in area and has an even depth of 0.8 to 0.9 m at the present time. A preceding anaerobic lagoon receives sewage from the village of Deshler. Two thousand residents and the town drainage contribute from 1.2 million liters to 2 million liters of sewage per day. An engineering or construction firm would evaluate the Deshler lagoon system negatively because it obviously is designed to accept a much larger volume. Retention of sewage in the system is from one to several months. The reason for such a capacity may have been a group of city fathers who were over-optimistic about the population growth of their village, or architects who were extremely conservative. Be that as it may, this lagoon is not subject to the regular ills and misfortunes of lagoon systems whose capacities are constructed close to the margin of input volumes.

The question of efficiency in lagoon systems needs to be debated no longer. A lagoon system with sufficient capacity can produce effluent of the highest quality (there are several studies which document this, but we will cite Schurr[1] and Schurr and Raum[2]). In comparative efficiency, a properly designed lagoon system will convert nutrients better than standard activated sludge or trickling filter systems. Lagoons are appropriate treatment methods for small towns, villages, animal feed lots and similar industrial uses. However, they are the system of choice only where land use and cost of land will permit their construction. Benefits of lagoons are low construction cost and low operational costs.

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There are two products of lagoon sewage treatment: the water, and a huge volume of plant and animal material. Both are subjects for our conference. Plant and animal biomass is quite innocuous from a public health standpoint. It is not offensive to the senses. If this biomass is allowed to leave with the effluent, however, it will eventually die and decay will release the nutrients downstream. We can not tolerate this eutrophication. Algal blooms in Lake Erie have nearly destroyed it for swimming and recreational boating because they are so negative aesthetically. With severe eutrophication, the algae supersaturate the water with O_2 from photosynthesis in the day, but there is zero oxygen because of respiration at night. Few fish can tolerate such conditions and even fewer eggs or embryos will survive.

The logical solution to this difficulty would be to remove the biomass and find a use for it. Many researchers have tried to utilize single celled algae from primary lagoons, but their efforts have been plagued with problems. The high yields of algal cultures tantalize physiologists. Verduin and Schmid[3] could document approximately 7000 grams of protein produced by Chlorella, per m^2 of surface, as compared with about 150 grams for traditional terrestrial crops. The difficulties in use of primary lagoon algae have been with harvesting where they clog pumps and filters. This would not be an insurmountable technical problem, but the population dynamics of the algae vary through the seasons and many species taste badly or are toxic. Dohms[4] has summarized the efforts to use algae for food. Since outstanding scientists have found so many problems with unicellular algae, we have studied the possibility of using multicellular forms, the flowering plants and invertebrates that grow in the terminal lagoon at Deshler. A following paper at this meeting by Dr. Bakaitis will evaluate vitamin content of biomass.

YIELD AND USE OF BIOMASS

Schurr[5] and Schurr[6] describe potential nutritional value of the invertebrates, while Dohms and Schurr[7] document the protein, lipid and carbohydrate content. Living biomass in the Deshler terminal lagoon is dominated by Potamogeton foliosus, a flowering plant which has several growth forms. In the terminal lagoon, the stems break and the plant blooms in large floating masses which continue to grow through the summer to a maximum crop in August, September and October. It forms a thick mat that eventually shades the bottom of the pond. Water content of the biomass was determined by lyophilization of preweighed samples (Dohms[4] & Bakaitis[8]). This rigorous drying of biomass showed an average percentage of water to be 87% (standard deviation 0.25) for 1971, 1972 and 1973. We took two samples in 1968, three sets in 1969, two sets in 1971 and one sample in 1973. The samples were blotted in newspaper, and air-dried in sunlight. The final consistency was the same as well-cured hay. Water content calculated from this method of drying was slightly above 84%. The method of drying is much less accurate than lyophilizing, but it more closely approximates the results from potential commercial treatment. For this reason, we will use 85% water in estimating productivity. Samples were raked into a plastic container, excess water was drained, and the biomass was packed into plastic bags to be frozen for later chemical analysis or determination of volume. Measurements of the floating biomass mat gave a calculated yield of wet weight comparable to sample volume.

A single crop for the terminal Deshler lagoon will yield 1596 cubic meters of dry biomass per hectare. Proper removal of the floating material will benefit growth, so that no shading of the undersurface can occur. We expect this would increase yield to over 2000 m^3 per hectare. Nutritional content of the biomass has been documented over the growing period by Dohms[4]. A typical sample for October was composed of 22% protein, 4% lipid, 40% carbohydrate and 34% ash (fractions rounded to nearest percent). Oxygen bomb calorimitry measured energy content per gram of the biomass at 3.86 Kcal to 3.92 Kcal with a standard error of 0.047 on five replicates. Sewage lagoon biomass, therefore, has considerable potential as a food resource. The high protein content becomes particularly significant because of the general shortage of protein. Foss[9] has summarized the information, from the international symposium on "New Sources of Protein Foods," as a world requirement for increased protein. This is 3,630,000 metric tons of new dry weight protein each year for the foreseeable future. Conventional agriculture and our normal fisheries will not produce this quantity (Schurr[5]). Biomass from sewage lagoons may offer another source of protein.

PROPOSED METHODS OF HARVEST

Methods of handling biomass have yet to be evaluated. The standard alfalfa drying plant should be able to dry and pellitize biomass. The consistency of biomass and wet alfalfa cut at night is similar. Removal from the lagoon is possible with a floating rake pulled to one end by cable and two electric motors. This would remove floating mats yet leave attached stems for continued growth. Biomass could be loaded for transportation with the same pick-up choppers used for wet alfalfa. These suggested methods of removal would enable harvesting at the best rate to promote plant growth. They also utilize the existing technology for alfalfa drying.

ALTERNATIVE USES

The actual disposal of biomass will be determined by the research which is in progress or has yet to be done. If there are no public health problems, sewage lagoon biomass could be used as a component in feed for several domestic animal species. It could even serve as a fractional part of meat substitutes in the human diet; I have eaten it and the taste is bland. Feeding of biomass would be particularly advantageous for animal feed lots, where it could be added to more conventional feeds. Animal manure could be converted to biomass in lagoons on the farm and then recycled through the animals again. If there should be evidence of pathogenic bacteria or viruses in the biomass, it may be necessary to mix the biomass with corn as ensilage. As is usual with this feed, the mixing of molasses with the biomass and corn would certainly increase its nutritional value. Fermentation and heating would probably destroy pathogens in the silo.

Should some unforeseen problem prevent feeding of biomass, then the material could be used to generate methanol as is described by Reed and Lerner[10]. Mixed-grade methanol can be produced cheaply from garbage, plant refuse, paper pulp wastes and biomass. Methanol can be used as a fuel additive to gasoline, where about 15% will increase octane, reduce air pollution and extend mileage in standard automobile engines. Methanol converters could generate fuel, from wastes and biomass, to be used in municipal vehicles and farm tractors.

A final possible use of biomass would be as a soil conditioner. Such use would not give as much monetary return as those mentioned above, but it would dispose of the material. Sweet clover is frequently planted and then ploughed under in order to improve soil fertility and texture. Whatever the use, it is necessary to remove biomass from lagoons so that it will not go out with effluent, die, decompose and release the nutrients in down-stream areas.

PROBLEMS

The use of biomass or water from lagoons will depend on their freedom from toxic substances. Pesticides, herbicides and industrial chemicals must not be present in significant levels. Cyanides are often released from factories as are lead, mercury, cadmium and selenium. Unexpected contaminants may be found; Robinson, Draper and Gelman[11] showed that copper sulfate fed to pigs resulted in sufficient copper in feces to inhibit nitrogen degradation. Radioactive elements are another possible hazard.

A serious public health concern is the fate of waterborne virus particles and pathogenic bacteria. Hicks[12], Saslaw[13] and Kurowski[14] show the potential danger. Several studies have been completed on lagoon systems and the pathogens they may harbor. There has been no well-controlled study of virus particles in a lagoon system having a reasonable retention time. We expect several scientific papers which speak to this problem because there is new commercial equipment now available for concentrating virus particles from large volumes of water (Wallis, Homma and Melnick[15]). The Deshler system seems to dispose of bacteria rather well (Schurr[1]). Kott[16] has stated that virus particles are attenuated in the lagoon system that he is testing. Nevertheless, it is necessary to have exaustive studies completed on lagoon systems which are properly loaded. It is appropriate that similar research be done on activated sludge and trickling filter systems as a comparison.

Problems May Be Caused By Members Of Our Profession

A lagoon system which is "efficient" from the standpoint of land use and cost, is frequently the type of system that concerns the public health officer, offends the senses and is an ecological abomination. We can evaluate lagoons as efficient in cost or loading as opposed to those which are efficient for their purpose. Our definition of efficiency is the second type; sewage treatment should convert wastes to materials or chemicals which can be removed during or after treatment so that the effluent is water of highest quality. Absolute standards of water quality can be met by trickling filter or activated sludge systems combined with flocculation and chemical treatment. The system which is in operation at Lake Tahoe would be an example of efficiency in our view (Abelson[17]). Cost of such a system is quite high. We differentiate efficiency of treatment from cost in our discussion because there is a tendency of some designers to mix apples and oranges, in their calculations, and the product is something that smells badly. It is wrong ethically and wrong from an engineering viewpoint to present a plan that mixes circa 60% waste conversion with construction costs that seem what the traffic will bear. It is also wrong to view the sewage system as a microcosm and then reject any responsibility for what leaves it. The public health officer who is only concerned with heavy chlorination of effluent is in error. Nothing may be alive in the outflow, but there is an indication that reactions may take place which produce chlorinated hydrocarbon residues that will be of public concern in food chains further down in the watershed. Chlorinated effluent will destroy stream ecology for miles. There are individuals with tunnel vision. If all bacteria and viruses are destroyed, they don't concern themselves with the chlorine, nitrates, nitrites, phosphates, etc. being dumped in our waterways. Ozone (actually ozonized air) would be a reasonable alternative to chlorination if the chemical were removed by aeration.

PLAN FOR INTEGRATED LAGOON AND STANDARD SEWAGE SYSTEMS

Many small towns have overloaded sewage plants. They may also have mixed storm drains and sanitary sewers. This places an intolerable burden on those responsible for proper treatment of sewage. Bowling Green State University responded to such conditions by offering land at no cost for the construction of 400 acres of lagoon system. The university also financed a comprehensive waste water study (E.C.C.[18]). Bowling Green has mixed storm and sanitary sewers, with a trickling filter sewage plant that is overloaded during rainfall. The proposed sewage lagoon system would receive all effluent during rainfall and treatment would progress through a chain of lagoons. Biomass would be removed from terminal lagoons. Effluent could be pumped back through the trickling filter system during dry periods. High loadings could be sent to the lagoons and then brought back to the standard plant. An efficient removal of phosphates could be achieved most economically.

The alternatives to this proposed combination of lagoons and trickling filter sewage treatment plant are as follows. The town must construct a greatly expanded sewage treatment plant. Design figures on plans show that even this expanded capacity will be unable to accept the volume during heavy rains and it will be necessary to bypass. Storm and sanitary sewers must be separated. We predict that federal regulations will become more stringent and storm water will be treated within a few years. Better quality standards on effluents will be mandated by the government. This will require a flocculation system being added to the new treatment plant.

It is difficult to estimate construction costs within the next few years because of inflation, energy shortages and land expense. However, we think the total cost of a new sewage treatment plant, plus separation of storm and sanitary sewers, plus treatment of storm water, plus special treatment required as standards increase, may reach the level of twenty million dollars. No useable product will be produced by this type of construction. Alternatively, with free land contributed by the university, a lagoon system could be added to the sewage plant at a cost of $400,000 to $600,000. A compromise treatment system composed of a new activated sludge system and a lagoon system would cost about $7 million and could meet the highest water quality standards. The town has 20,000 residents listed on the last census, but this included all students living off campus. Actual residents are about 15,000. Those with taxable incomes are much fewer. It becomes obvious that there must be alternatives to standard sewage treatment because the average American community simply doesn't have the tax base to build an activated sludge or trickling filter system that will meet the highest water quality standards. Increased welfare costs, fire protection, police protection, education, mental health programs, etc., etc. will all have priority over sewage treatment. Clean water will be an impossible goal unless we can find ways to treat sewage at moderate cost.

SUMMARY

We suggest that the proper prescription for optimum sewage treatment with reasonable costs is as follows. Villages, small towns and animal feed lots would be appropriate for lagoon systems if land cost is reasonable. Loading should be moderate so that terminal lagoons will be aerobic and will produce biomass from plant and animal growth. The biomass should be removed and commercially used as described in this paper. Whenever possible, the lagoons should be combined with standard sewage treatment systems in order to obtain the highest water quality standards in treatment. The biomass should be recycled close to the site of production. This may be used as animal feed. We do not discount the use for humans if it could meet pure food and drug standards. We may find the proper fate of biomass would be a conversion to methanol to be used as an additive to gasoline. Biomass may be disposed of as a soil conditioner. Its removal from lagoons, however, is a necessity if we are to reach the ideal of pure water as effluent.

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PART 3 AQUACULTURE

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ANALYSIS OF SEWAGE LAGOON BIOMASS WATER SOLUBLE VITAMINS BY MICROBIOLOGICAL TECHNIQUES

by

Nancy M. Bakaitis*

Eutrophication of surface water resources, due to enrichment from fertilizer runoff, municipal sewage effluent and other sources, has led to great economic losses and environmental damage. If the vast growths of vascular plants and algae, which occur under these conditions, could be harvested economically, mechanical removal would be an attractive alternative to the chemical treatments now employed.

Water weeds have been suggested as a potential source of nutrition for livestock by Boyd[1] and Little.[2] Recent work on cultivation of aquatic plants in sewage lagoons to remove pollutants and produce a clean effluent has had considerable success in the United States and in Asia, according to Oswald and Golueke[3] and McGarry and Tongasame.[4] This concept of sewage treatment and food production has also been tested successfully by Ryther[5] using aquatic plants in marine systems.

The sewage lagoon system of Deshler, Ohio was studied from 1971 to 1973 to determine the nature of the life forms commonly present and their nutritional value. The possibilities of sewage treatment and water purification with the production of a useful product were explored by several workers including Dohms[6], Schurr[7-8], and Verduin.[9]

The Deshler sewage lagoon system consists of two ponds with a depth of approximately one meter. The system handles all of the sewage from a community of 2000. Pond One receives raw sewage through a submerged line at the rate of from 300,000 to 500,000 gallons per day, depending on rainfall. Anaerobic digestion of sewage occurs in this nine acre pond. Pond Two is thirteen acres in area and is aerobic. The water leaves Pond One through two corner outlets and enters Pond Two, which in turn, discharges into a small stream. Material from Pond Two was studied.

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Pond Two biomass consists of floating plant material in which Potamogeton foliosus Raf. predominates and has associated with it many aquatic insects at various life cycle stages, aquatic annelids, mites, and snails. The plant material forms a thick floating mat extending many feet from the shore toward the center of the pond. Sampling of the biomass was done by standard random sample techniques with samples being of 5 to 10 liters in drained volume. Samples were taken at various intervals during August and September each year when the material was present in significant quantities. Samples were lypholized and frozen.

Analysis of the water soluble vitamin content of the biomass was conducted to determine if it was comparable to common animal feeds as a vitamin source. Thiamine, riboflavin, niacin, pantothenic acid, B_{12}, total B_6, biotin, choline, and beta-carotene were selected for study based on the requirements of chickens and swine. Choline was determined using the method outlined by Sebrell and Harris.[10] Beta-carotene was found to be present using the procedure of György and Pearson.[11] All other vitamins were assayed using the preferred microbiological techniques of the Association of Official Agricultural Chemists[12], the Association of Vitamin Chemists[13], or the United States Pharmocopiae.[14] The content of each of the vitamins studied did not vary significantly during the growing season or from one year to the next so that the data was pooled and a mean vitamin content for each vitamin is presented.

Results indicate that the sewage lagoon biomass vitamin content compares favorably with common feeds, such as, alfalfa, ear corn, dried barley, sugar beet pulp, and solvent extracted soybean hulls. The amount of riboflavin present in the biomass is superior to these feeds; and, the amounts of biotin, choline, niacin, pantothenic acid, and thiamine are comparable. The total B_6 content of the sewage lagoon biomass is low in comparison to these feeds and would need to be augmented if it was the sole B_6 source in chicken rations. A summary of these findings is presented in Table I.

Analysis of subsamples of biomass material taken in 1971 were completed by Dohms[6] for protein, carbohydrate, lipid, and mineral content. The amounts of carbohydrate present was reported as 3.89 Kcal/g dry weight of material; and, the protein content was found to be 17.9 per cent. These values also compare quite favorably with dried alfalfa meal, corn, barley, soybean hulls, and sugar beet pulp protein and carbohydrate content. The mineral content of the biomass was also found to compare favorably with these feeds being higher in calcium and zinc, but lower in manganese content. A summary of these findings is in Table II.

Therefore, the sewage lagoon biomass from the Deshler, Ohio aerobic sewage lagoon compares favorably in protein, fat, carbohydrate, mineral, and vitamin content with commercial feeds. Regardless of the source,

Table I. Vitamin Content of Feeds Compared to Sewage Lagoon Biomass Mean Vitamin Content for 1971 to 1973[a]

Vitamin	SLB[b]	Feed[c]	Feed[d]	Feed[e]	Feed[f]	Feed[g]
Biotin	0.36	--	0.20	--	0.05	0.32
B_{12}	0.10	--	--	--	--	--
Choline	1020	1550	1030	829	550	2743
Niacin	30.00	41.90	57.40	16.30	20.00	26.80
Pantothenic Acid	13.18	20.90	6.50	1.50	5.00	14.50
Total B_6	1.35	6.50	2.90	--	5.00	8.00
Riboflavin	19.81	10.60	2.00	0.70	1.10	3.30
Thiamine	5.00	3.00	5.10	0.40	--	6.60

a. Expressed as mg/kg dry weight of material.
b. Sewage Lagoon Biomass mean assayed value based on 1971, 1972 and 1973 samples.
c. Alfalfa, Medicago sativa, aerial portion, dehydrated.
d. Barley, Hordeum vulgare, dried grain.
e. Sugar Beet, Beta saccharifer, dehydrated pulp.
f. Corn, Zea mays, ground ears.
g. Soybean, Glycine max, seed meal , whole, solvent extracted and ground, 7 per cent fiber.
Data for c.-g. from NRC, Nutrient Requirements of Poultry,[15] 1971. pp. 24-37. Table 13. Composition of Some Common Poultry Feeds.

Table II. Mineral Content of Feeds Compared To Sewage Lagoon Biomass Assayed Mineral Content [a]

Mineral	SLB[b]	Feed[c]	Feed[d]	Feed[e]	Feed[f]
Sodium	0.29	0.07	0.02	0.01	0.01
Phosphorous	1.03	0.22	0.42	0.28	0.62
Calcium	3.44	1.23	0.08	0.04	0.26
Potassium	1.39	2.33	0.53	0.53	2.02
Manganese (ppm)	464.0	29.00	16.30	13.00	45.50
Magnesium	0.41	0.29	0.12	0.15	0.28
Iron (ppm)	1601.00	0.03[a]	0.01[a]	0.01[a]	0.02[a]
Zinc (ppm)	50.00	20.00	15.30	9.00	45.00
Copper (ppm)	10.00	10.40	7.60	7.70	--

a. Expressed as per cent dry weight unless otherwise indicated.
b. Sewage Lagoon Biomass, Dohms,[6]1972. A Nutrient Analysis of Sewage Lagoon Biomass. Table VI.
c. Alfalfa, *Medicago sativa*, aerial portion, dehydrated.
d. Barley, *Hordeum vulgare*, dried grain.
e. Sugar Beet, *Beta saccharifer*, dehydrated pulp.
f. Corn, *Zea mays*, ground ears.
Data for c.-f. from NRC, Nutrient Requirements of Poultry,[15] 1971. pp. 24-37. Table 13. Composition of Some Common Poultry Feeds.

if nutritionally valuable feeds can be found, they will be employed in animal feeds. The only significant limiting factors are cost and public health. The cost of sewage lagoon biomass will be determined by the expense of harvesting and transport, since it is a "waste product" of sewage treatment at the present time. A positive economic factor is the present necessity of removing this material from effluents in order to release clean, treated water. Such removal prevents eutrophication in down-stream areas of the watershed. Since lagoons are the least expensive method of treating sewage from villages, small towns, and animal feed lots, the removal of biomass will be the final requirement of lagoon treatment in the future. With removal of biomass, lagoons also become the most efficient method of sewage treatment. Removal of biological oxygen demand, nitrates, nitrites, and phosphates is considerably better than trickling filter or activated sludge systems operating at optimum efficiency.

Removal and utilization of biomass in feeds would enable our society to reap a triple benefit. Lagoon treatment could be employed more generally in small communities and for animal feedlots. Secondly, the biomass could be used for animal feeds near the location of production. Actual recycling of nutrients would be possible for small geographic areas and for individual farms. Thirdly, we could approach the ecological ideal of confining nutrients to the areas where we want to promote growth, while avoiding dispersal of nutrients to locations where we consider them harmful to the habitat.

At this time, all studies conducted on the Deshler sewage biomass have produced results which indicate that biomass would be an excellent feed supplement. Before a final evaluation of biomass as a feed source can be made, information must be obtained on the fate of virus particles and bacteria in a well-operated sewage lagoon system. The combinations of sewage lagoon biomass with other components of livestock rations and the effect on growth need to be documented in actual feeding experiments. It will also be necessary to design methods of harvesting and drying the biomass.

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FEED AND FIBER FROM EFFLUENT - GROWN WATER HYACINTH

by

L.O. Bagnall, T.deS. Furman, J.F. Hentges,Jr.,
W.J. Nolan and R.L. Shirley*

SUMMARY

Water hyacinth was used to remove nutrients, primarily nitrogen and phosphorous, from secondary treated sewage effluent; some of the plants were subsequently ensiled or dried and fed to sheep and cattle and some were pulped to make paper. The irregularly harvested pond removed 10% of the nitrogen and phosphorous from the effluent, only 10% of which could be accounted for in the plant tissues. Cattle and sheep readily ate processed water hyacinth in complete diets and remained in good health, but did not utilize the nutrients as well as nutrients in a land forage, coastal Bermudagrass. The primary feeding value is as sources of energy, mineral elements and roughage for ruminants. Paper can be made from water hyacinth, but production cost is uneconomically high. Compost may be the best use, having the highest value and lowest processing cost.

INTRODUCTION

Fertilization by wastewater often encourages excessive growth of some plant species in waterways, reducing the availability of the water for more desirable uses. The focusof this conference is on how to manage these fertilizing effluents to some beneficial ends. One possible means to this end is to manage the growth and utilization of one of the problem species, water hyacinth (Eichhornia crassipes).

Water Hyacinth

Holm, Weldon and Blackburn[1] estimate water hyacinth caused losses of $43 million in the Southeastern United States in 1956 and that control

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programs cost millions of dollars. Though not the only cause of this undesirable excessive growth, over-nutrification of the water is a contributing factor.

Water hyacinth is a large free-floating plant with an attractive lavender flower and shiny bright-green leaves on long petioles. Uncrowded plants, particularly in shallow water and full sunlight, have bulbular float petioles about 8 inches long, whereas crowded plants produce elongate pwtioles up to 50 inches long[2]. The plants reproduce from stolons and, less importantly, seeds. Penfound and Earle[2] report that the number of water hyacinth plants doubled every 11.2 to 15.0 days in field observations and that the edge of a water hyacinth mat extends 2 feet per month. Bock[3] reported a 50% weight increase in 13 days, and Knipling West and Haller[4] observed mass growth rates of 50% in 10.4 days. Stand densities of 125 to 184 tons per acre have been reported[2].

Water hyacinth grows most rapidly in water temperature from 28 to 30 C[4] and pH from 4.0 to 8.0[5], and ceases to grow when water temperature is above 40 C or below 10 C. Water hyacinth is killed when the tip of the rhizome is frozen[2]. The plant has little tolerance for salt water and will only grow in fresh to faintly brackish water. The plants are normally free-floating but, if stranded by receding water, will root in mud and survive. Phosphorous is limiting to growth in concentrations below 0.1 ppm and is consumed in luxury amounts at higher concentrations[6]. Plants usually exhibit lower root-to-shoot ratios as water nutrification increases.

Because of their habit of vegetative reproduction from stolons, individual plants remain tied together in a floating mat. In confined waters, the mat spreads until it covers all the water. Penfound and Earle[2] report that dissolved oxygen under mats ranged from 0.1 to 1.5 ppm, depending on mat density, when nearby open water contained 4.0 ppm oxygen. The oxygen depression is caused primarily by decay of detritus from the mat and in turn causes dramatic changes in the types and quantities of life in adjacent areas.

Wastewater and Water Hyacinth

Sheffield[7] passed extended aeration effluent through a water hyacinth pond with a detention time of 10 days and observed 81% reduction of ammonia nitrogen. No reduction in nitrate nitrogen was observed until anaerobiosis was established at 42 days, after which reduction rose to 81%. Orthophosphates were reduced 51% during the first month of operation, but reduction fell to 20% after that, as decaying plant detritus released accumulated phosphorous back to the water.

Clock[8] found that a water hyacinth system removed 39 to 94% of the total nitrogen from extended aeration effluent when the detention time was five days, with best reduction coming during the period of most vigorous growth. Average nitrate reduction was 61%. Phosphates were reduced by 60% during periods of vigorous growth, but increased during dormancy.

Steward[9] projected that an acre of water hyacinth can take up the nitrogen production of 130 to 595 people from secondary sewage effluent when growth rate ranges from 294 to 1340 tons per acre-year. At the same growth the phosphorous production of 40 to 180 people could be absorbed.

Miner, Wooten and Dodd[10] grew water hyacinth on anaerobic swine lagoon effluent in Iowa and found the system to reduce Kjeldahl nitrogen 95%, ammonia nitrogen 96%, phosphate 82% and COD 88% in 102 days detention in pools 18 inches deep, and recommended that depth for application.

Food, Feed, Fiber and Water Hyacinth

Boyd[11] found the composition of naturally growing water hyacinth to be as shown in Table 1. He noted that the composition varies considerably with location and season, but concluded that, chemically, it was suitable for cattle feed. Water hyacinth would be unsuitable for human food because of its high fiber content. It could be fractionated to yield leaf protein and other non-fibrous components.

Cattle have been observed eating water hyacinth leaves, especially when other types of forage were scarce. Hentges[12] reported that cattle accepted limited amounts of fresh and sun-dried chopped water hyacinth, but refused some plant parts and stale feed. He fed dehydrated water hyacinth to cattle at high levels of their diets and found that, though the animals only maintained their weight, the material was not toxic; he noted that the quality of the feed was poor due to a combination of source and processing.

Most paper is made from wood, but other plant materials have been tested as pulp sources. Paper-making from water hyacinth has been suggested and tried in the past, but thorough, scientific data is not in the literature.

Processing

The water hyacinth must be removed from the effluent to remove the nutrients and put them to use. For most uses the plants must be processed to reduce bulk and weight, retard or enhance decomposition, and to improve acceptance and utilization. Growth on an artificial pond should be more consistent in quality and quantity than at natural sites, and controlled harvesting should be easier, important factors in commercial application. The non-conventional harvesters required are available.

The harvested plants' characteristics and some of its proposed uses resemble those of some traditional land forages. Forage processing practices, including chopping, ensiling, drying and pelleting, should be applicable with modification. Casselman, et. al.[13] explored the use of presses for reducing the high initial moisture content of some tropical forages. Pirie[14] applied maceration and pressing to extraction of pro-

Table 1. Composition of Water Hyacinth[a]

	%, dry basis (except as noted)
Dry Matter, % wet basis	5.9
Crude Protein	16
Nitrogen	2.5
Cellulose	28
Available Carbohydrate	7.8
Ether Extract	3.5
Ash	17
Phosphorous	0.42
Calcium	1.0
Potassium	4.4
Magnesium	1.1
Energy, kcal/g dry	3.8

a. from Boyd[11]

tein from freen plants. In both cases, the resulting juice was rich in protein, minerals and pigments. Pirie describes ways that these valuable components can be recovered, including pH adjustment, aging and heating which cause coagulation or precipitation. The required technology for utilization is available and needs only to be adapted to the crop.

OBJECTIVES

How much nitrogen and phosphates can water hyacinth remove from secondary treated sewage effluent? How do pool geometry, season, and detention time affect removal? How should plant harvest be managed?

What is the cost of producing compost? What is its value? Is there a market?

Will cattle readily consume processed water hyacinth? Can they utilize the nutrients the processed water hyacinth contain? Will they perform well on processed water hyacinth? Are there health-related problems from eating water hyacinth? What is the cost of producing processed water hyacinth feed? What is its value? Is there a market?

Can paper be made from water hyacinth? What are the production requirements of such paper? What are the characteristics of the pulp and paper? What is the value of the paper and the cost of its production?

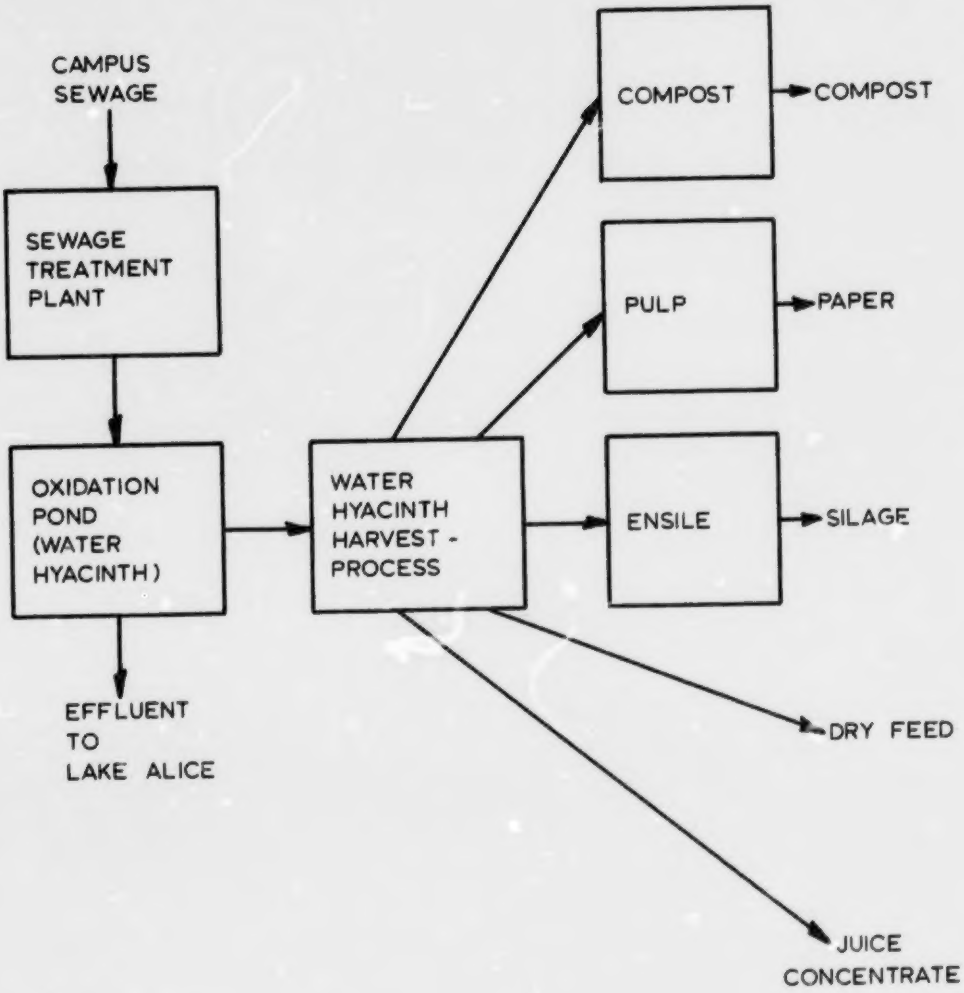
What product is most feasible, economically?

PROCEDURES

Water hyacinth was grown on extended aeration secondary treated sewage effluent, harvested, and processed to produce a range of products as shown in the overall system schematic in Figure 1. Loosely coordinated individual projects in nutrient removal, paper-making, processing and animal feeding used the same pool of water hyacinth.

Water hyacinth was established on one of the oxidation ponds at the University of Florida Campus Sewage Treatment Plant in early April, 1972. Seven bushels of plants were collected from Lake Alice, on the University of Florida campus about a half mile downstream from the sewage plant, and placed in the 7400 ft^2 pond, where they initially covered an estimated 75 ft^2. Surface coverage was determined periodically until the pond was covered. In late May, nine 3x3 ft pens were built in an adjacent pond and plants were placed in them for mass growth rate determinations.

Influent and effluent were sampled automatically and analyzed for Kjeldahl nitrogen, ammonia nitrogen, nitrate nitrogen, orthophosphate, and total phosphate. Samples for dissolved oxygen determinations were taken near the center of the pond at 6 inches below the surface, mid-depth


CAMPUS
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HYACINTH
HARVEST -
PROCESS
COMPOST
COMPOST
PULP
PAPER
ENSILE
SILAGE
DRY FEED
JUICE
CONCENTRATE

Figure 1. Water Hyacinth Effluent Nutrient Removal - Utilization Scheme

and 6 inches above the bottom.

The pond was operated throughout most of the test with a 15-hour detention time, much shorter than any reported previously in nutrient uptake tests with water hyacinth. During the last 2½ months of the test, from early February to mid-April, 1973, detention times of 6 to 24 hours were used, to establish the effect of detention time in this range.

The system shown in Figure 2, combining conventional and unconventional forage processes, was used to convert aquatic plants to a variety of products. It consisted of harvesting, chopping, pressing, ensiling, drying, pelleting and juice solids recovery operations and produced compost, pulping stock, silage, dry feed and a feed concentrate.

Plants were harvested irregularly throughout the summer and fall of 1972 by hand or with a small conveyor. Harvested plants were chopped with a slightly modified forage harvestor.

Compost was produced from whole and chopped plants by storing wet plants aerobically for 1 to 6 months, then drying and grinding. Compost was evaluated by mixing with other soil materials and growing plants in pots.

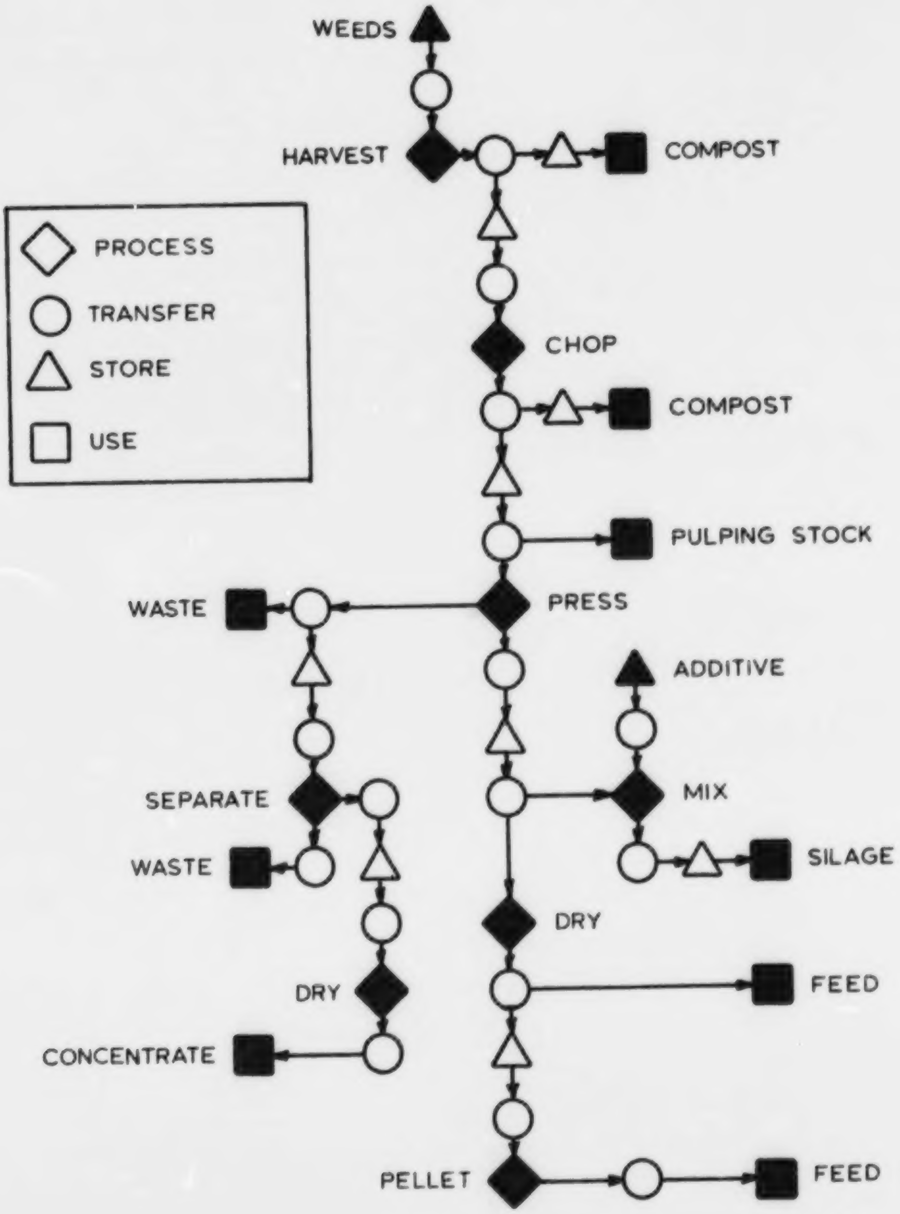
Large quantities of chopped plants for feed production tests were pressed in the 12-inch Vincent press used by Casselman, et.al.[13]. Smaller lots were pressed in the eight-inch and nine-inch presses built by Bagnall[15]. Methods of recovering nutrients, particularly protein, from the juice were explored.

Most of the effluent-grown water hyacinth was ensiled. The pressed water hyacinth was mixed with up to 4% dried citrus pulp or cracked corn and up to 1% standard cane molasses as free carbohydrate sources prior to ensiling. Lots of 220 lb were stored for 21 to 60 days in polyethylene-lined barrels with trapped drains. Lots of about 3000 pounds were stored in four-feet diameter by 8-feet long asphalt-lined currugated culverts set on end.

Some of the pressed water hyacinth was dried in a static bed at 125 F to 140 F and stored as coarsely ground feed. Water hyacinth from other sources was dried on other types of dryers, including an instrumented high-temperature rotary dehydrator. Drying characteristics and costs can be projected from the combined data.

One lot of processed water hyacinth was pelleted through a 3/8x2-inch die in a flat plate pellet mill. Densities and durabilities were found and observations made regarding production rate and energy requirement.

Whole plants, chopped plants, pressed plant residue, pressed juice, dried plant products, silage and mixed feeds were analyzed for moisture and nutrients, primarily crude protein and ash. In some cases, more


WEEDS
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WASTE
PRESS
ADDITIVE
SEPARATE
MIX
WASTE
SILAGE
DRY
DRY
FEED
CONCENTRATE
PELLET
FEED

Figure 2. Aquatic Weed Harvesting - Processing System

detailed analyses, primarily of the ash, were made.

Animal acceptability of water hyacinth products was established by placing them before the animals for several days, either as the only feed available or in competition with other feeds, and observing order of choice and quantity consumed. Feed was weighed before feeding and leftovers weighed back and replaced each day to minimize effects of deterioration, especially with silage.

Yearling steers were fed dried water hyacinth, dried hydrilla (*Hydrilla verticillata*) and coastal Bermudagrass (*Cynodon dactylon*) as 33% of the organic matter in complete pelleted diets to determine acceptability, as measure by voluntary intake, and utilization of nutrients, as measured by balance of intake and collected excrement. Sheep and cattle were fed water hyacinth silage from the culvert and tower silos, alone and with supplementary dietary components, to determine voluntary intake and nutrient utilization. Coarsely ground dried water hyacinth press residue was included as 10% of cattle diets for comparison with cottonseed hulls and sugarcane bagasse pellets as roughage sources in a 112-day feeding trial.

Health of all animals was observed during all trials and selected animals were slaughtered and examined in detail.

Whole plants and elongate petioles only were chopped and used for pulping. The pulping system is shown in Figure 3. The chopped plants were passed through an attrition mill to reduce the plants to very small particles and break the physical bond between fiber bundles and pith. The pulp was washed on a travelling screen by high pressure water spray, and the clean fiber bundles were dried and stored. The fiber was pulped by cooking in experimental digesters using four different processes shown in Table 2. Pulp fiber strength was developed and fibers separated by beating. The pulp was tested for freeness, a measure of drainage rate, and test handsheets were formed. Tear, burst and breaking strengths were found.

RESULTS AND DISCUSSION

An area growth curve of water hyacinth in the oxidation pond is shown in Figure 4. Time for doubling of area in the central part of the curve, following establishment of the stand and before crowding, is 6.2 days. This rate of growth is about twice that observed by Penfound and Earle[2]. When 60% of the water hyacinth was harvested in August, recovery was very slow until the remaining mat was broken up; peripheral length must be maximized to assure maximum growth.

Time for 50% increase in wet weight was 7 days, indicating a growth rate 43% higher than that observed by Knipling, West and Haller[4] and 86% faster than that observed by Bock[3]. Fertilization and other conditions existing in the pond are conducive to higher than normal growth rates.

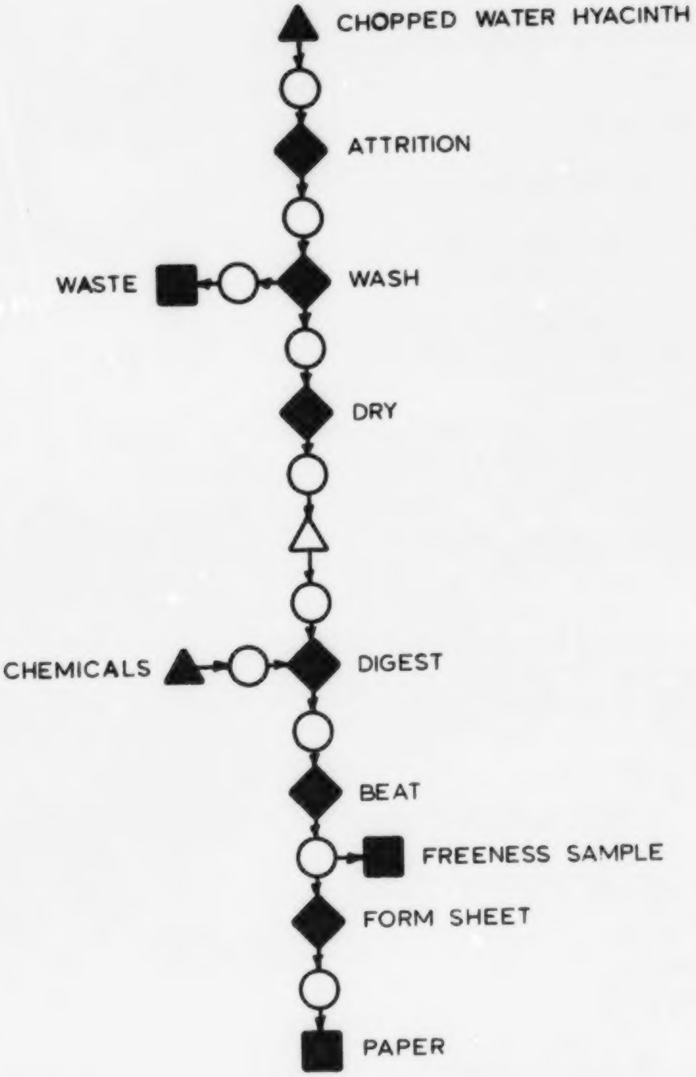
CHOPPED WATER HYACINTH
ATTRITION
WASTE
WASH
DRY
CHEMICALS
DIGEST
BEAT
FREENESS SAMPLE
FORM SHEET
PAPER

Figure 3. Water Hyacinth Pulping Process

Table 2. Water Hyacinth Paper-Making Processes

Chemical concentration	Liquor Ratio	Temperature C
70 g/l sodium sulfite	7:1	135 - 172
25 g/l sodium hydroxide	7:1	121 - 148
36.6 g/l sodium bisulfite + 10-35 g/l sodium sulfite	12:1	156 - 162
35 g/l equivalent Na_2O, 25% sulfidity sodium hydroxide + sodium sulfide (kraft process)	12:1	121 - 156

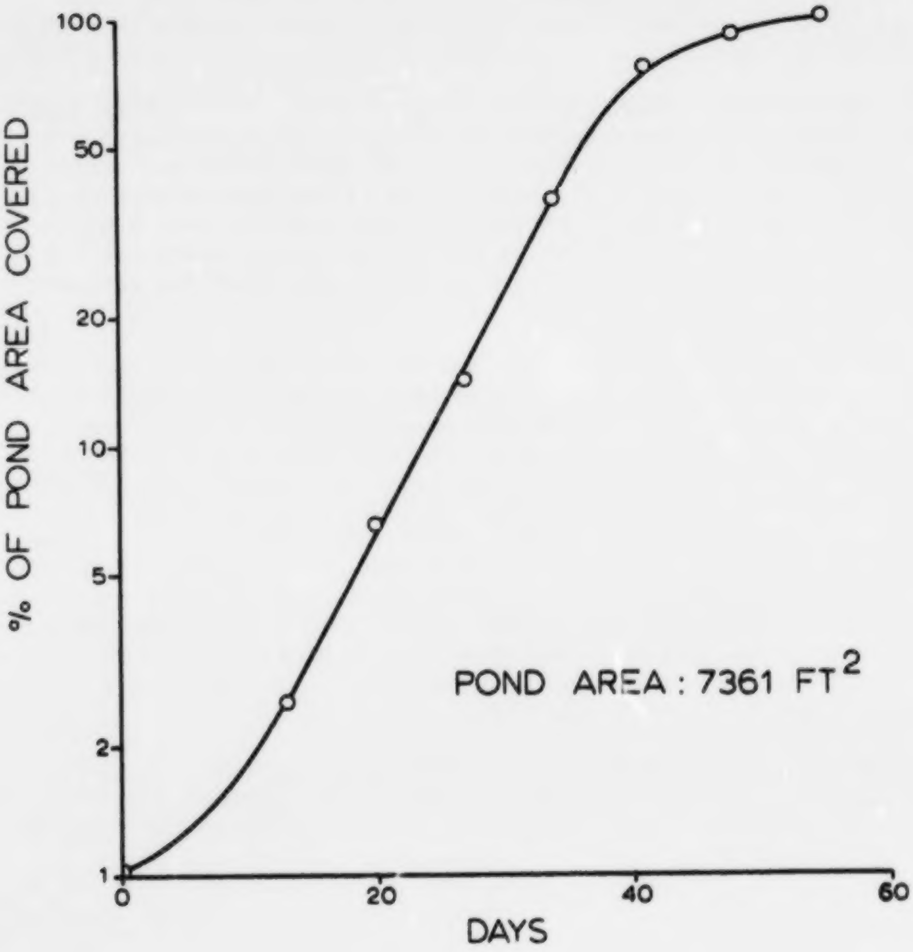

Figure 4. Water Hyacinth Area Growth in Sewage Treatment Plant Effluent Oxidation Pond

Dissolved oxygen concentrations in the pond ranged from 0.95 ppm near the bottom to 3.46 ppm near the surface; anaerobiosis was not an important factor in nitrogen reduction. Total reductions of nitrogen and phosphorous for the year are shown in Table 3 with average influent and effluent concentrations. Nitrogen and phosphorous removal during the test are shown in Figure 5. Removal of nurients was much lower than reported by other investigators, at least partly attributable to much shorter detention time.

The plant roots extended downward only about 2 inches into the 54-inch deep pond, exposing the roots to only 4% of the water. The nutrients must be carried to the roots by turbulence, of which there was little, and diffusion. A shallower pool, such as the 18 inches recommended by Miner, et. al.[10] would probably be more satisfactory for this application. During most of the test period the plants were crowded and not growing vigorously, so managed harvesting might have improved performance.

Nitrogen and phosphorous balances for the first ten months of operation of the pond are given in Table 4. Stand density, based on weight and area of plants removed for ensiling in August, was 111 tons per acre. Nitrogen and phosphorous in the plants are based on stand density, area, known moisture and nitrogen contents and estimated phosphorous content. Only about 10% of the two nutrients was removed, and only about 10% of the removed nutrients can be accounted for in the plant tissues. Some of the missing nutrients may be in the detritus on the bottom of the pond. With a mass growth rate of 50% every week, 1/3 of the plants could be removed each week, for a total of 218 tons in the ten months of testing. This management scheme would have removed 476 pounds of nitrogen and 127 pounds of phosphorous, instead of the 24 and 6.5 pounds, respectively, shown in the table.

Effect of short detention times on nitrogen and phosphate removal is shown in Figure 6. Removal is generally greater at greater detention times but data are very irregular, possibly due to weather and transient effects, and inconclusive.

Harvesting - Processing

Harvest rates of over 4 tons per hour per foot of width have been observed for flat-wire-belt conveyors with reels[16]. A conveyor uses less than 0.33 horsepower-hours per ton. Cost of harvesting was $2.13 per wet ton; costs at a fixed site with reliable machinery could be as little as 25% of that figure.

A forage chopping cylinder reduces water hyacinth satisfactorily, but feed and discharge systems of most choppers must be modified to give a satisfactory capacity[17]. A 16-inch wide cylinder chops almost 2 tons per hour per inch of cylinder width and required a minimum of 0.18 horsepower-hours per ton.

Table 3. Nutrient Removal for the 12-Month Study Period

	Average Influent ppm	Average Effluent ppm	% Removal
Total Nitrogen	12.11	10.98	9.3
Kjeldahl Nitrogen	5.57	4.80	13.8
Ammonia Nitrogen	3.88	3.31	14.6
Nitrate Nitrogen	6.54	6.18	5.3
Total Phosphates	4.19	3.81	8.0
Orthophosphates	3.18	3.03	4.8

Table 4. Nutrient Balance in Water Hyacinth - Covered Oxidation Pond

	Total Nitrogen		Total Phosphorous	
	lb	%	lb	%
Entering pond in 10 months	6670		2310	
Leaving pond in 10 months	6040		2100	
Removal	630	9.4	210	9.1
Removed in Plants, August	24	0.4	6.5	0.3
Inventory in Plants, February	41	0.7	11	0.5
Missing	565	8.4	193	8.2

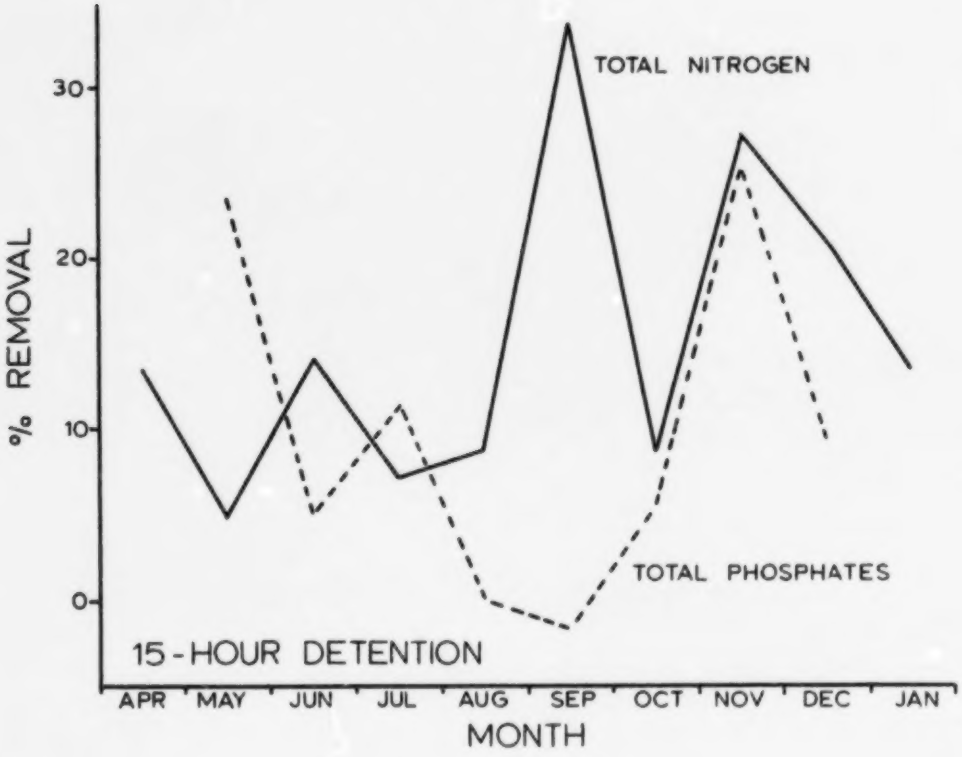

Figure 5. Nutrient Removal in Water Hyacinth - Covered Oxidation Pond

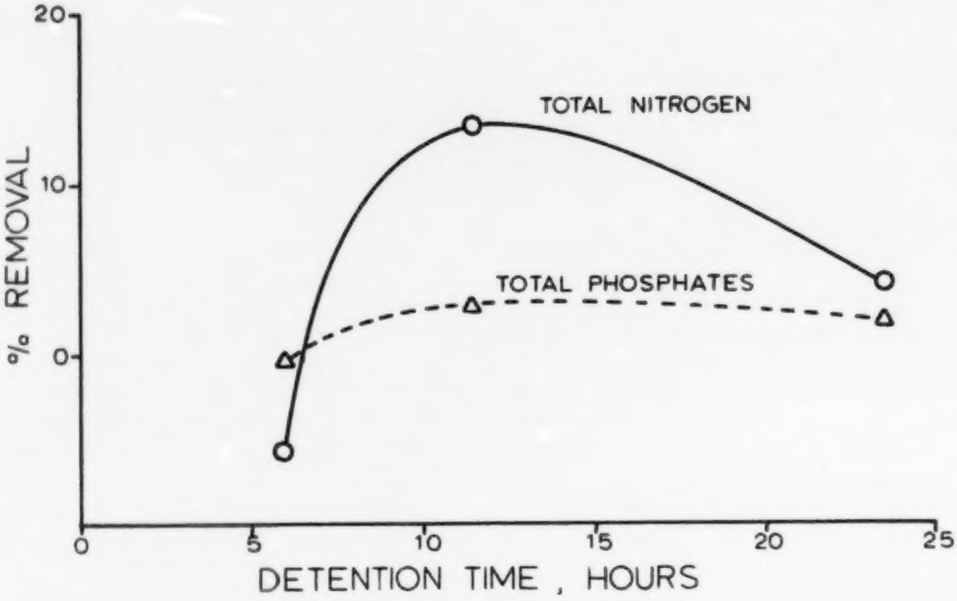

Figure 6. Effect of Detention Time on Nutrient Removal in a Water Hyacinth - Covered Oxidation Pond

A light-weight, decreasing-volume screw press can readily remove over 75% of the water from water hyacinth and is reasonably tolerant of uneven loading and trash[18]. A 12-inch press can easily process 12 tons per hour and a 16-inch press should be able to process 28 tons per hour. Estimated energy requirement is 2.5 horsepower-hours per wet ton or 4 horsepower-hours per ton of water expressed.

Relatively sophisticated combinations of chemical, thermal and mechanical juice solids recovery can be used on a fixed site with enough space. Recoveries of up to 63% were achieved in the laboratory[19] and could be exceeded with improvement in equipment and technique.

Good quality silage can be made from pressed water hyacinth by adding about 4% cracked corn or dried citrus pulp and storing anaerobically[20]. If moisture content of the pressed water hyacinth is below 85%, drainage will be negligible. Excellent silage was made in 21 days and 60-day silage was very acceptable.

Water hyacinth must be dried thoroughly and quickly to make dry feed. Whole plants in static beds dry slowly and with only 13% efficiency. Pressed plants dry only 10% faster than whole plants and 60% more efficiently, but the principal benefit of pressing is the large, rapid water reduction with low energy input and the small gain in drying performance is a secondary benefit. Without agglomeration of fines, performance would have been much better. A 6000-pound-per-hour rotary dehydrator dried 6800 pounds of pressed water hyacinth per hour from 88 to 22% moisture, using 1500 BTU per pound of water evaporated; the higher efficiency is partially attributable to fluffing of the product before and during drying.

Pure dried water hyacinth flows poorly and is very frictional and abrasive, causing very low pelleting rates and very high energy requirement. Mixed feeds containing water hyacinth, corn and soybean meal can be pelleted at high rates and reasonable power requirements but pellet density and durability are lower.

Material balance for the complete processing system producing 100 pounds of dried water hyacinth is shown in Figure 7. Larger or smaller systems can be scaled proportionally.

Estimated processing costs per dry ton of product are $12.12 for harvesting, $1.26 for chopping, $5.25 for pressing, and $26.60 for drying. Resulting product costs on a bone dry ton basis are $13.38 for compost at 78% moisture, $33.03 for silage at 80% moisture and $45.23 for dry feed at 10% moisture. Bases for these costs are a 100-wet-ton-per-hour intermittant operation, 20% solids loss between harvest and product, $3.00 labor, $0.25 diesel fuel, $0.20 bunker C oil for drying, and typical machine costs and efficiencies. Neglected were costs of buildings and transfer and storage equipment. The mechanical operations are capital-intensive, while drying is energy intensive. Capital and labor costs per unit of production would be smaller for larger and continuous

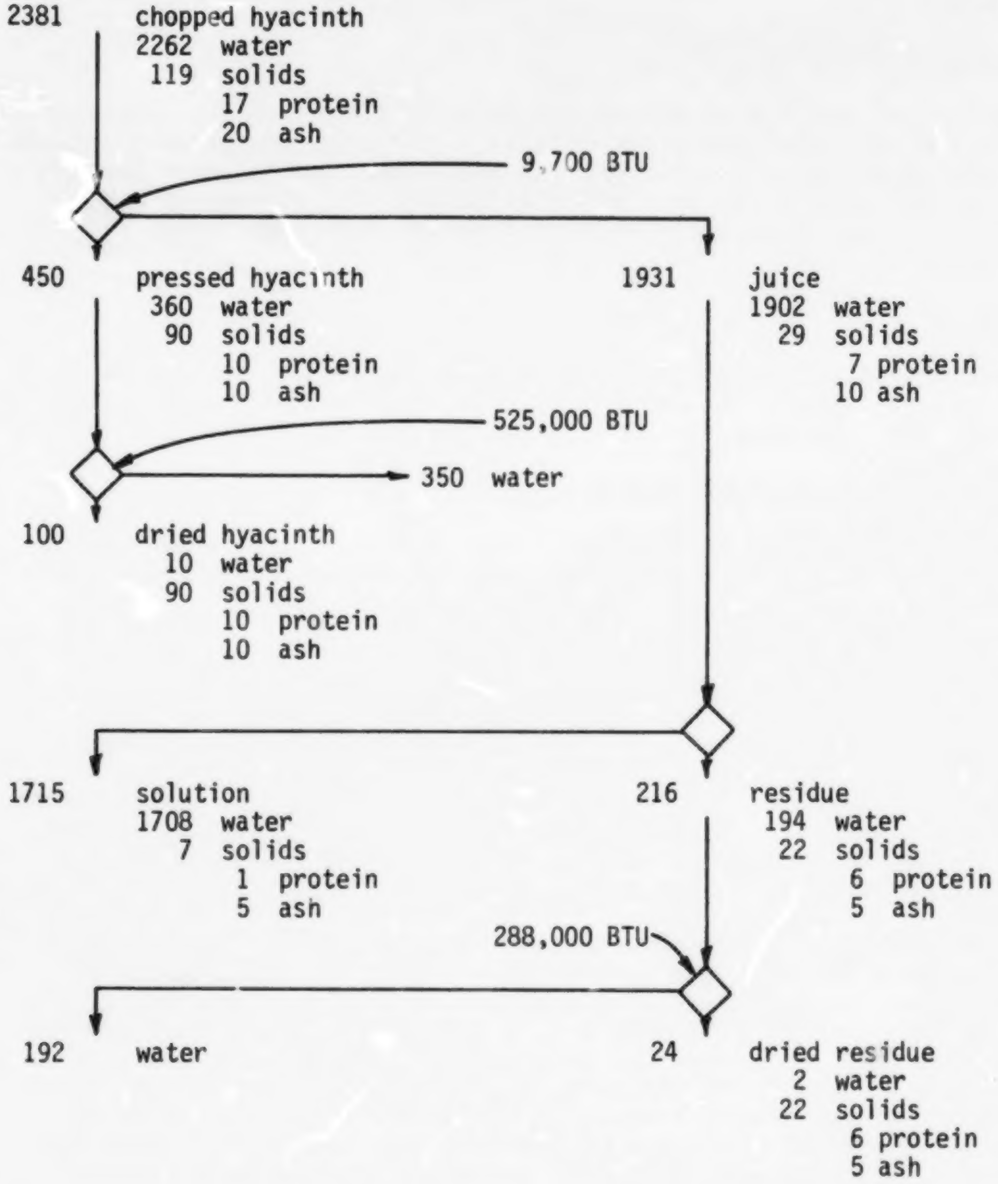
2381
chopped hyacinth
2262 water
119 solids
17 protein
20 ash
9,700 BTU
450
pressed hyacinth
360 water
90 solids
10 protein
10 ash
1931
juice
1902 water
29 solids
7 protein
10 ash
525,000 BTU
350 water
100
dried hyacinth
10 water
90 solids
10 protein
10 ash
1715
solution
1708 water
7 solids
1 protein
5 ash
216
residue
194 water
22 solids
6 protein
5 ash
288,000 BTU
192
water
24
dried residue
2 water
22 solids
6 protein
5 ash

Figure 7. Process Material Balance for 100 Pounds of Dried Water Hyacinth

operations.

Composition

Analyses of the chopped and pressed water hyacinth from the oxidation pond and Lake Alice are given in Table 5. Moisture and protein contents of the oxidation pond water hyacinth are higher than those of the lake water hyacinth, in response to fertilization. Ash content is high in both and should be considered in diet design, both with regard to quantity and balance.

Compost

Composted water hyacinth holds water unusually well. Plants grow poorly in pure compost, but perform satisfactorily in sand:compost ratios around 3:1. Estimated market value of the compost is about $46 per ton.

Utilization by Ruminant Animals

Cattle and sheep voluntarily consumed diets containing processed water hyacinth. When only pressed dried water hyacinth was offered to steers, consumption was less than 1% of body weight; consumption was increased to 1% by adding 30% molasses and to 1.5% by pelleting[12]. Steers consumed 2.1% of their body weight of a pelleted diet containing dried water hyacinth as 33% of the organic matter; consumption was not significantly lower than that of a similar coastal Bermudagrass diet[22]. Cattle accepted most water hyacinth silages immediately, but consistently refused silages with no dry carbohydrate additive. The most acceptable silages had the highest additive (dried citrus pulp or corn) levels, lowest pH, lowest ash, and highest protein[23]. Oxidation pond water hyacinth silage was accepted better than Lake Alice water hyacinth silage.

Digestibility by cattle and sheep of diets containing processed water hyacinth varied with level of water hyacinth in the diet, source of water hyacinth and method of processing. Dry matter digestibility in dried water hyacinth was half that in Bermudagrass hay, apparently reflecting nutrient loss in the press juice[22]; however, there were no differences between the two diets for net retention and apparent absorption of ten mineral elements[24]. Digestibility of organic matter and crude protein was higher in oxidation pond than in lake water hyacinth silage, as shown in Table 6, but was highest in pangolagrass silage[23].

Animal performance was best when level of water hyacinth, on a dry organic matter basis, was less than 25% of complete diets for cattle and sheep. All animals remained in good health throughout all experiments. Levels of potential toxicants, namely oxalates, nitrates, tannins, cyanides and dicoumarin apparently were within safe ranges as no toxicity was observed[26].

Table 5. Composition of Processed Water Hyacinth

	Oxidation Pond		Lake		
	Chopped	Pressed	Chopped	Pressed	Silage***
Dry Matter, % wet basis	4.6	11.7	5.0	9.6	18.6
Crude Protein, % dry basis	14.8	12.7	11.3	10.6	9.1
Ash, % dry basis	22.5	13.0	23.0	17.3	11.2
Calcium, % dry basis				2.28	2.4
Phosphorous, % dry basis				0.4*	0.4
Potassium, % dry basis				2.0*	4.7
Sand-silica, % dry basis				5.9*	---
Crude fiber, % dry basis				---	21.7
Cellulose, % dry basis				26.1**	----
Ether Extract, % dry matter				-----	1.9
Gross energy, local per gram				-----	4.0

*from Stephens[24]

**from Salveson[22]

***from Kiflewahid[25]

Table 6. Digestibility of Nutrients in Water Hyacinth Silage by Sheep[a]

	Organic Matter Digestibility %	Crude Protein Digestibility %
Oxidation Pond Water Hyacinth + DCP[b]	48	51
Lake Alice Water Hyacinth + DCP	40	53
Pangolagrass + DCP	54	76

[a] from Baldwin[23]

[b] Dried citrus pulp added at ensiling time

In feedlot and digestion trials, dried water hyacinths satisfactorily replaced the conventional roughages, cottonseed hulls and pelleted sugarcane bagasse, at levels from 10 to 20% of complete high energy diets.[25]

All criteria measured showed processed water hyacinth has a replacement value of at least equal to cottonseed hulls and sugarcane bagasse pellets. Current market price of these products is $40 to $45 per ton.

Fiber

After attrition and washing, 18 to 20% of the plant material was recovered as clean fiber.[26] In the sulfite process and sulfite-bisulfite process, pulp yield was as high as 80 to 88% of the fiber placed in the digester; the highly alkaline hydroxide and kraft processes yielded only 40 to 50%. Paper strengths were low when yield was above 65 to 75%. Cooked fiber bundles had to be beaten to separate them into individual fibers.

Mechanical characteristics of the papers are shown in Table 7. Freeness of the water hyacinth pulp is much lower than that of pine kraft pulp and reduces the freeness of blends. Hyacinth pulp drained to paper on a screen in 5 to 10 minutes, while pine kraft pulp requires only about 30 seconds; a given plant would have only 6% the capacity using water hyacinth pulp as it would have with pine kraft pulp. Slow drainage also produces a paper of non-uniform strength and texture. Water hyacinth paper had usually high shrinkage and was dark and dirty-appearing.

Tear factor decreases and breaking length increases with increasing water hyacinth content in blends and were highest for the alkaline processes and lowest for the sulfite-bisulfite process. Burst factor increased with increasing water hyacinth content, was highest for kraft process and lowest for the sulfite-bisulfite process. Use of peticles only instead of the whole plants slightly improved breaking length and tear factor but decreased burst factor and freeness. Breaking length and burst factor are higher than those of pine kraft, but low tear factor would make the paper unacceptable for packaging and low freeness and yield would make it uneconomical to produce.

CONCLUSIONS AND RECOMMENDATIONS

A water hyacinth covered pond with a detention time of over 10 hours removed 9% of the nitrogen and 8% of the phosphorous from secondary treated sewage effluent. Removal is increased to 80% and 60%, respectively by increasing detention time to 5 days. Low plant vigor, caused by crowding and low temperature, and excessively deep water reduce removal. Only 10% of the removed nutrients were found in the plant tissue. Plants should be harvested regularly at the rate of 25 to 33% per week to maintain maximum growth and nutrient uptake.

Table 7. Mechanical Characteristics of Water Hyacinth Pulps and And Papers at 10-Minute Beating Time[a]

	Freeness ml	Tear Factor	Breaking Length m	Burst Factor
Pine kraft	720	205	4300	26
65% pine kraft, 35% water hyacinth	160	130	9600	52
50% pine kraft, 50% water hyacinth	90	95	10000	54
Sulfite, whole water hyacinth	40	28	7200	53
Sulfite, water hyacinth peticles	20	30	7900	42
Sulfite-bisulfite, water hyacinth	45	15	6600	40
Hydroxide, water hyacinth	40	32	8700	52
Kraft, water hyacinth	30	35	>11000	>75

[a] from Nolan[26]

Compost can be produced from water hyacinth at a cost of about $3.00 per ton and sold for about $46 per ton. A readily developable market to nurserymen and home gardeners is available.

Cattle and sheep readily consumed water hyacinth silage and will consume dried water hyacinth up to 25% of a complete diet. Processed water hyacinths had nutritional value in cattle and sheep diets primarily as a source of energy, mineral elements and roughage. Animal performance and health on diets containing processed water hyacinths were satisfactory. Estimated production cost of dry feed is $40.70 per ton and silage is $6.62 per ton. Estimated value as dry feed is $40 to $45 per ton. Market development depends on consistency of quantity and quality of supply and price.

Paper can be made from water hyacinth, but yield and freeness are low, making production uneconomical. The paper has good breking and burst strength but low tear strength. Because of low production rate, production cost would be too high to be competetive.

Compost appears to be the most feasible product, followed by cattle roughage.

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THE AVAILABILITY OF DAPHNIA FOR WATER QUALITY IMPROVEMENT AND AS AN ANIMAL FOOD SOURCE

By Ray Dinges*

INTRODUCTION

Modern aerobic biological wastewater treatment facilities are capable of reducing biochemical oxygen demand of their influents by some 85 to 90 per cent. The biological activity occurring in such plants has been described by Dinges[1] as the fermentation phase of biological treatment and is characterized by external digestion facilitated primarily by solubilization of organic material by bacterial enzymes. See Figure 1. Secondary to the fermentation phase is the consumption phase which is characterized by internal digestion. The predaceous consumption phase essentially occurs in receiving waters. It should be understood that the three phases outlined signify dominant features and are not separate and apart but overlap to different degrees dependent upon water quality and physical conditions of the environment. Insects, for example, may be present in all three phases.

This paper deals with what has been termed the consumption phase, and specifically, a Daphnia dominated community. In recent years there has been considerable interest developed regarding the possibilities of expanding conventional processes of biological wastewater treatment for reduction of remaining organic fractions by utilizing higher life forms. Uhlmann[2], Loedloff[3], Erlich[4], Scheithauer and Bick[5], and DeWitt and Candland[6] have all investigated the potential of utilizing Daphnia to attain improvement of wastewater effluent. Kryutchkova[7] discussed energy flows in wastewater stabilization ponds as related to consumption of particulates by zooplankton. Greer and Ziebell[8] utilized asiatic clams Corbicula for reducing the content of phosphorus from stabilization pond effluent through their consumption and deposition of algae and other suspended matter. Development of a wastewater treatment process making marine filtering organisms available to consume particulates from treated effluent is continuing at the Woods Hole Oceanographic Institution in Massachusetts.

DESCRIPTION OF DAPHNIA

As reported by Ward and Whipple[9], Daphnia habitats include temporary pools, small ponds and lakes. Daphnia are common aquatic

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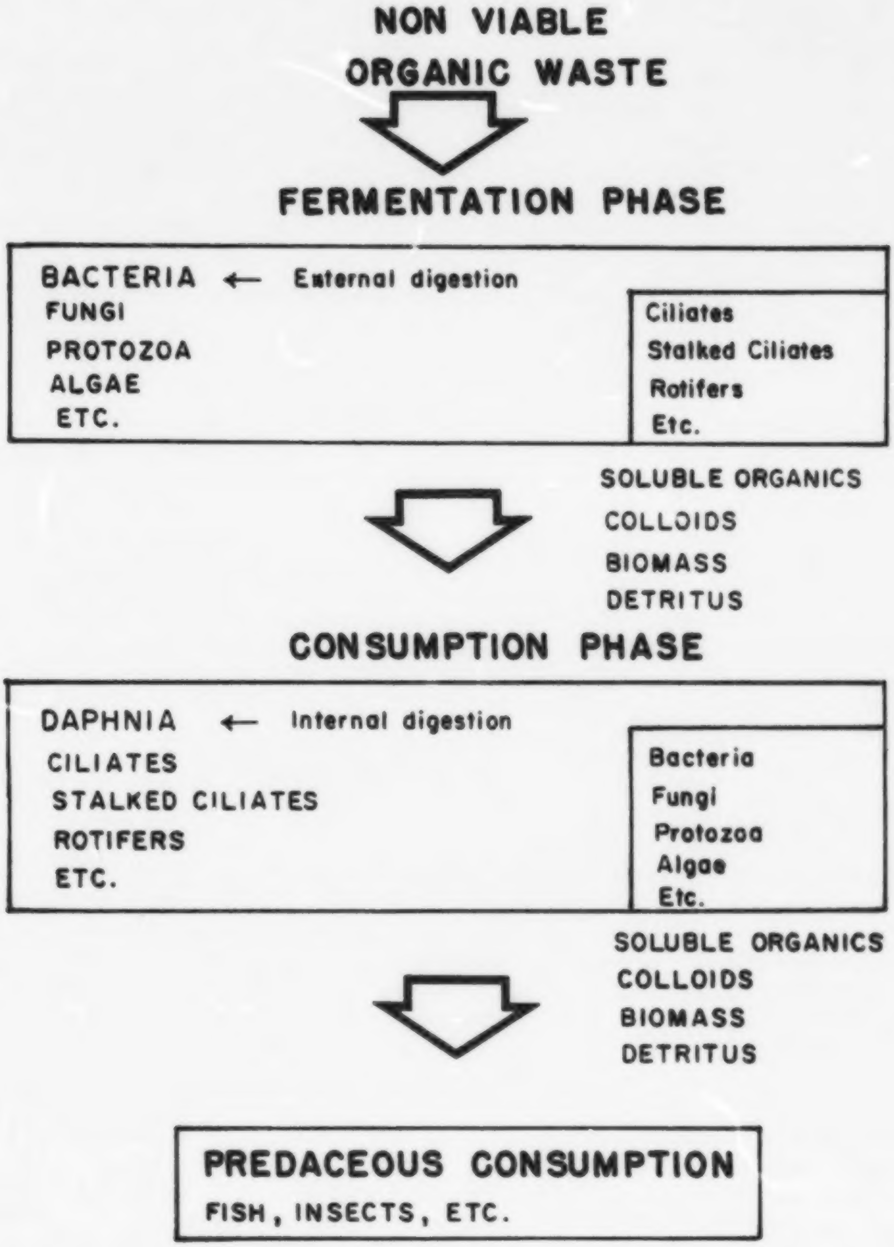
Figure 1

BIOLOGICAL ORGANIC STABILIZATION


NON VIABLE ORGANIC WASTE
FERMENTATION PHASE
BACTERIA ← External digestion
FUNGI
PROTOZOA
ALGAE
ETC.
Ciliates
Stalked Ciliates
Rotifers
Etc.
SOLUBLE ORGANICS
COLLOIDS
BIOMASS
DETRITUS
CONSUMPTION PHASE
DAPHNIA ← Internal digestion
CILIATES
STALKED CILIATES
ROTIFERS
ETC.
Bacteria
Fungi
Protozoa
Algae
Etc.
SOLUBLE ORGANICS
COLLOIDS
BIOMASS
DETRITUS
PREDACEOUS CONSUMPTION
FISH, INSECTS, ETC.

crustaceans about 1/8 inch in length and feed upon algae, bacteria, protozoa and debris. Food particles of a proper size are apparently filtered from water and consumed in a rather indiscriminate manner.

Daphnia exhibit both sexual and parthenogenic reproduction. A population will consist almost entirely of parthenogenic females when environmental conditions are satisfactory. A parthenogenic female may produce 30-40 new *Daphnia* every two days. Male *Daphnia* appear when environmental conditions become less than optimal and sexual eggs are produced. Two eggs are enclosed in a dense, durable structure called the **ephippium**. Sexual egg production serves to assure survival of *Daphnia* when habitat conditions again become favorable. The life span of *Daphnia* is about one month. Two species found in Texas wastewater stabilization ponds were *Daphnia pulex* and *Daphnia similis*. *Daphnia similis* is shown in Figure 2.

ENVIRONMENTAL FACTORS

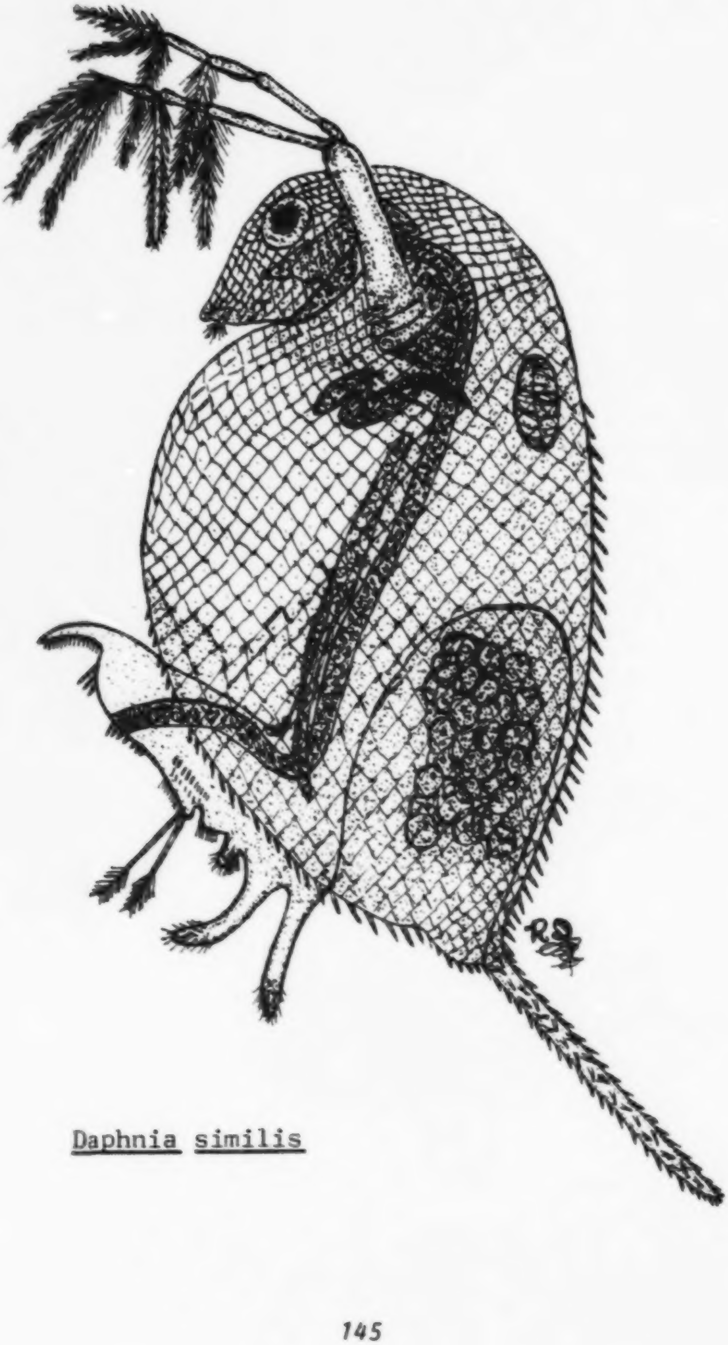
Stabilization ponds supporting *Daphnia* populations were noted to have effluents that were macroscopically clear and stable. With cooperation of field staffs of the Department and the Texas Water Quality Board, as well as municipal employees and the chemists of our Central Laboratories, a concerted effort was made during 1970 to 1973 to identify ponds supporting *Daphnia* populations. Occurrence of *Daphnia* was found to be quite uncommon, as they were present in only 29 pond systems out of the 470 domestic wastewater treatment facilities in Texas utilizing ponds. Field surveys were conducted to evaluate environmental factors affecting *Daphnia* with the intent of establishing, if possible, a continuous culture for effluent improvement. See Figure 3.

LIGHT

Observations made during the first year of the field studies revealed quite clearly that *Daphnia* population pulses in stabilization ponds were seasonal phenomenon. *Daphnia* populations appeared during the early winter months and disappeared in late spring. Figure 4 represents available sunshine and air temperature data on a monthly average basis as indicated by weather records for Austin, Texas. It was postulated that a *Daphnia* population pulse was either dictated by temperature, or photoperiod.

After having made a review of the literature and conducting further studies, it was concluded that, in Texas, photoperiod is the dominant environmental factor affecting *Daphnia* in stabilization ponds. The consequence of long periods of bright

Figure 2



Daphnia similis

Figure 3

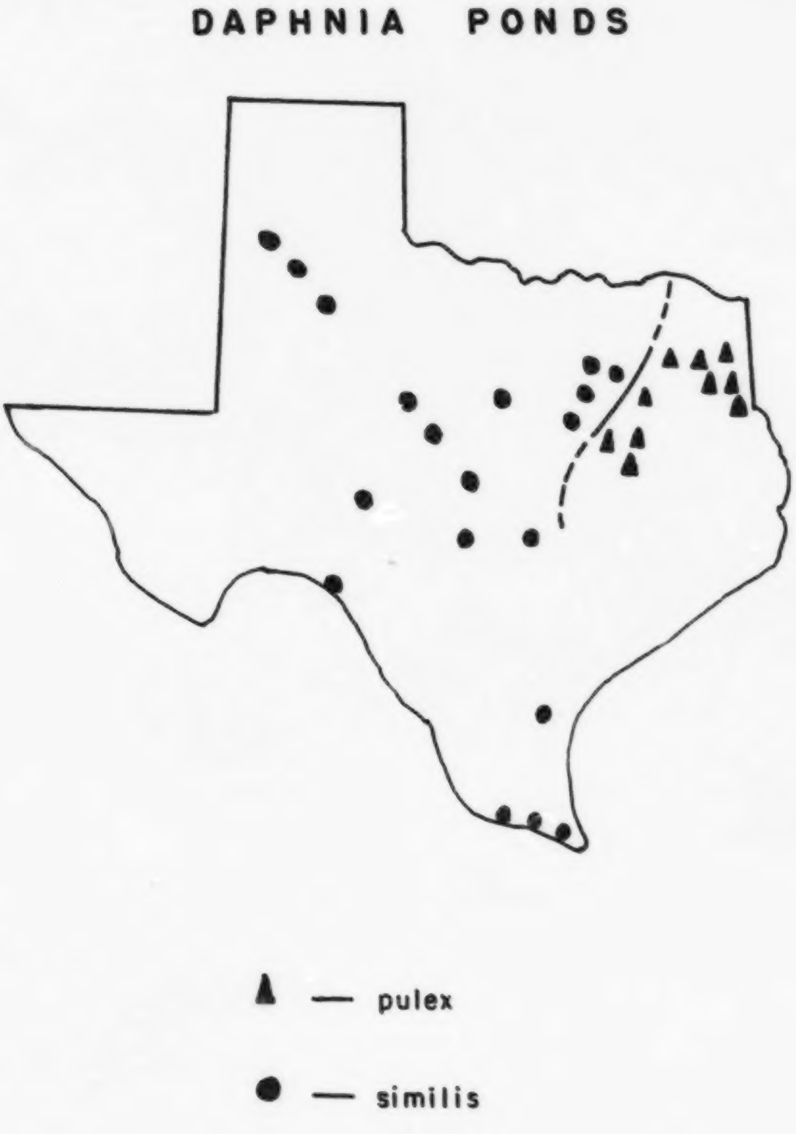
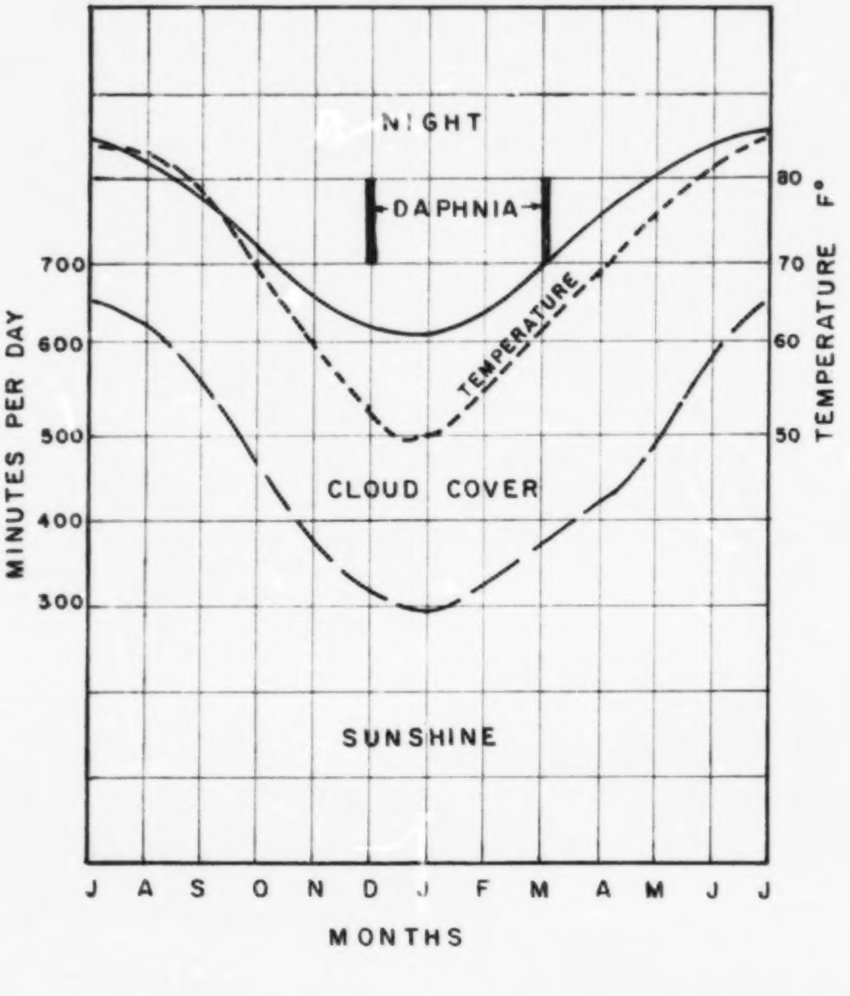

DAPHNIA PONDS
▲ — pulex
● — similis

Figure 4

AVAILABLE SUNSHINE



sunlight is an excessive production of phytoplanktonic algae in ponds which results in high pH caused by algal uptake of available free carbon dioxide and the subsequent shift in the carbonate equilibrium at the expense of bicarbonates. Ammonia is almost always present in Texas stabilization ponds. As early as 1934, Chipman[10], demonstrated toxicity of ammonia to Daphnia at elevated pH levels.

TEMPERATURE

Ammonia toxicity results from dissociation of ammonia, which is a function of both pH and temperature, with ammonia becoming more toxic with an increase in temperature. Stahl and May[11], and others have shown that microstratification occurs in shallow stabilization ponds as a result of high temperature and lack of wind induced mixing. Stagnant lower layers in a stratified pond often are anaerobic and provide suitable environments for sulphate reducing bacteria. Warm water temperature enhances growth rate of the bacteria and sulphides produced are quite toxic. Scheithauer and Bick[5] have stated that Daphnia can tolerate up to 3.0 ppm of hydrogen sulphide, but prefer a level less than 0.4 ppm.

Several observers have attributed demise of Daphnia populations to water temperature increase. Algae free Daphnia cultures were maintained in shallow, outdoor concrete containers exposed to full sunlight during the past three summers. Water temperatures recorded daily in mid-afternoon during the summer of 1970 in an exposed culture container 14 inches in depth were typically in the upper 80's F°. Monthly mean, maximum and minimum surface water temperatures of Texas ponds during the period from 1967 to 1973 are depicted in Figure 5. It appears logical to assume that endemic strains of pulex and similis are able to withstand normal water temperatures in Texas stabilization ponds.

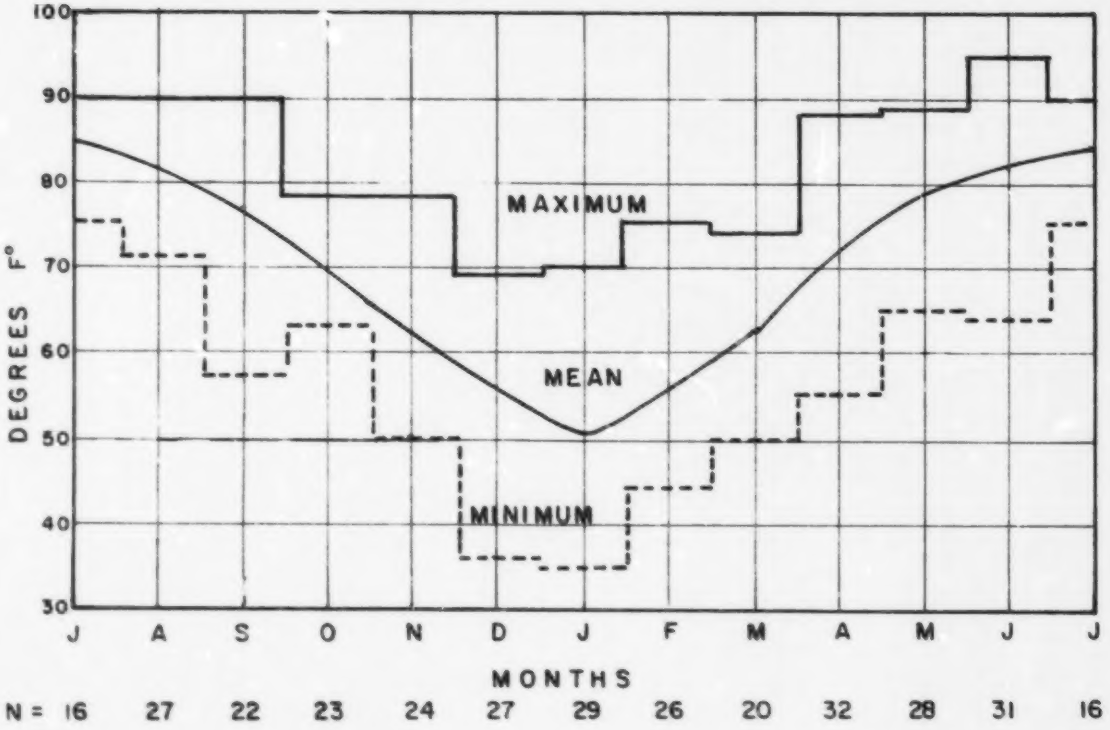
DEPTH

The physical characteristic most common to ponds with Daphnia populations was that of water depth. The usual depth of Texas stabilization ponds is 3 feet. Almost all ponds supporting Daphnia populations had depths varying from 5 to 15 feet. It is believed that lower layers of a deep pond have reduced pH levels suitable as an environment for Daphnia provided that sufficient dissolved oxygen for respiratory needs is present. Abnormal pond depth usually resulted from:

1. Deep ponds provided to serve as irrigation water holding basins.
2. Terrain considerations - Pond systems constructed on slopes or in uneven areas that made it uneconomic to level bottom.

Figure 5

TEXAS POND TEMPERATURES



3. Air-Aqua - Standard depth of 10 feet.

Several pond systems with *Daphnia* populations reported in the literature also were more than 3 feet in depth. Shallow *Daphnia* ponds mentioned were mixed by recirculation, or aeration.

MINERAL

Taub and Dollar[12], in devising a compatible media for concurrent culture of *Daphnia pulex* and *Chlorella*, reported that certain ionic relationships resulted in *Daphnia* mortality. They concluded that chloride should be the dominant anion and that a sodium to potassium ratio of less than 10:1 could result in toxic conditions. In addition, nitrate toxicity was variable, but decreased with an increase in chloride. They further concluded that chloride content should always exceed that of nitrate and nitrate level be kept as low as consistent to algae growth.

Mineral content and ionic relationships in *Daphnia* pond waters, therefore, assumed importance, as a serious handicap would exist in using these animals for effluent clarification if certain types of waters should preclude their production. Water samples were collected for mineral analyses from all ponds supporting *Daphnia*. On an average basis, the dominant anion found in *Daphnia pulex* pond waters was bicarbonate, but at times chloride was dominant. In waters of *Daphnia similis* ponds, chloride was usually the dominant anion, but on occasions bicarbonate was dominant. The lowest sodium-potassium ratio found in *Daphnia pulex* pond waters was 6.5:1, with a low of 4.7:1 in *Daphnia similis* pond waters. In most instances, there was a great difference between chloride and nitrate levels, with the chloride ion being dominant. However, on two occasions, nitrate levels in excess of chloride levels were found in waters of a pond supporting *Daphnia pulex*. The highest nitrate level found in *Daphnia pulex* pond water was 82 ppm and 55 ppm in *Daphnia similis* pond water. Results of field investigations indicate that mineral composition of waters in Texas wastewater stabilization ponds is suitable for *Daphnia* production. Mineral content of pond waters was found to determine dominance between *Daphnia pulex* and *Daphnia similis*, with *pulex* being restricted to relative soft, iron bearing waters of East Texas, whereas *similis* existed in hard, mineralized waters found in West Texas. Table 1 presents observed mean and extreme mineral levels in *pulex* and *similis* ponds. An overlap in range occurs on any individual mineral parameter between *similis* and *pulex* pond waters, but means and maximums exhibit significant disparity.

Table 1

Mineral Levels in 19 Daphnia similis Ponds N=70

ppm	Minimum	Maximum	Mean
Calcium	13	186	61
Magnesium	3	70	23
Sodium	59	630	291
Potassium	3	33	15
Bicarbonate	133	620	329
Sulphate	28	580	218
Chloride	53	790	276
Dissolved Solids	437	2,490	1,254
Hardness	80	660	249
Total Alkalinity	170	510	276

Mineral Levels in 10 Daphnia pulex Ponds N=21

ppm	Minimum	Maximum	Mean
Calcium	6	56	18
Magnesium	2	11	5
Sodium	55	225	111
Potassium*	2	12	7
Bicarbonate	31	400	183
Sulphate	16	85	48
Chloride	40	160	80
Dissolved Solids	308	800	496
Hardness	27	172	76
Total Alkalinity	25	328	155

* N=16

BIOLOGICAL

Fish probably would represent the most significant predator on Daphnia, as Daphnia are recognized as excellent fish food organisms. In Texas, Dinges[13] found that five fish species are potential inhabitants of wastewater stabilization ponds, or in otherwise dry stream courses receiving flow of stabilization pond effluent.

1. Mosquito Fish - Gambusia spp.
2. Warmouth - Chaenobryttus coronarius
3. Golden Shiner Minnow - Notemigonus crysoleucas
4. Southern Black Bullhead - Ictaluras melas
5. Carp - Cyprinus carpio

Of the fishes listed above, only mosquito fish were ever noted to occur in ponds supporting Daphnia populations, and then only in small numbers. Few ponds in Texas are known to support fish populations other than Gambusia, although almost all systems have been reported to have had fish introduced into the ponds. Several insects would be potential Daphnia predators. Of these, probably diving beetles Dytiscidae would be most important. Both the larvae and adults of this insect are very efficient predators in the aquatic environment. Diving beetles could possibly retard initial development of a Daphnia population, especially if they were present in large numbers, but Daphnia productive capacity is so great that a population would soon expand beyond the point where predation would make much of an impression.

Daphnia are not alone in clarification of water. The rotifers Brachionus and the stalked ciliates Vorticella were often noted as bionts on Daphnia and both feed upon suspended organic matter. Among other metazoa recorded were Ostracoda, Amphipoda, Mollusca, Acari, Insecta, Moina and other varieties of Branchipoda all contributing to the stabilization of organic material.

DAPHNIA CULTURE

Waters in a Daphnia culture unit are maintained in a clear, clean condition. Uhlmann[2] termed this condition as a klarewasserstadium, or clear water stage. In a klarewasserstadium, the population is in a state of semi-starvation with males being present and sex eggs evident. Daphnia populations tend to become balanced with available food supply and population growth rate is restricted by presence of sexual animals and a reduction in parthenogenic birth rates. A klarewasserstadium would be maintained if the objective of culture is production of high quality effluent. The population would be fed at a rate in excess of their dietary requirements in order to maintain continual parthenogenic reproduction at an advanced level if biomass production for commercial purposes is intended.

CULTURE UNIT

A small pond was constructed by the City of Giddings, Texas, to serve as a Daphnia culture unit at their Northside wastewater treatment plant, which consists of an Imhoff Tank and three stabilization ponds in series. A portion of effluent from the final pond was diverted to the culture unit. The outlet from the pond was provided with a concrete, broad crested, rectangular weir eight feet in length and with a crest width of six inches to reduce outflow velocity and washout of Daphnia. (Dinges[14] has prepared a paper on biological considerations of stabilization pond design for enhancement of zooplankton production, which is too lengthy to discuss here).

The first study phase was an investigation of the effectiveness of pH control by the regulation of pond exposure to sunlight and the resulting reduction of algae growth. The second phase evaluated both pH control by chemical addition and sulphide reduction through the mixing of pond waters by diffused air.

EFFLUENT QUALITY

Effluent quality results of samples collected weekly during the first run are presented in Table 2 and results of samples collected weekly during the second run are presented in Table 3.

Table 2

Mean Effluent Quality of _Daphnia_ Culture Unit
1-6-72 to 3-23-72

Detention - 11 days N=10 Loading - 35 lbs. BOD$_5$/acre/day

	Influent	Effluent	% Reduction
BOD$_5$, ppm	45.4	14.2	68.7
COD, ppm	271.1	90.6	66.6
VSS, ppm	77.2	10.8	86.0
Total coliforms	32,860.	9,160.	72.1
Fecal coliforms	6,880.	84.	98.7

Table 3

Controlled Operational Period of _Daphnia_ Culture Unit
2-27-73 to 4-9-73

Detention Time - 11 days Loading - 43.6 lbs. BOD$_5$/acre/day

Date		Influent, ppm			Effluent, ppm	
	BOD$_5$	COD	VSS	BOD$_5$	COD	VSS
February 27	90	245	179	7	60	8
March 6	80	310	121	7	60	11
8	105	320	142	9	90	8
13	25	75	27	<0.5	60	8
16	30	115	29	13	65	9
19	30	45	33	11	90	3
21	40	110	56	13.5	25	5
27	40	110	58	25	70	41*
April 3	85	160	63	12	95	19
9	50	95	76	20	85	18
Mean	57.5	158.5	78.4	11.8	70	13

Percent Reductions - BOD$_5$ - 79, COD - 55, VSS - 83

* Rotifer bloom in plant pond and passed through unit.

The BOD_5 remaining in the effluent from a Daphnia culture unit could be expected to approach the level of soluble BOD_5 of the influent. Soluble nutrient minerals were not removed in passage through the culture unit. Phosphate levels remained about the same and nitrates and organic bound iron increased.
See Figure 6 and Figure 7.

LOADING

Maximum permissible hydraulic and organic loading and minimum detention time on the culture unit were not determined. It is believed that a unit with a 5-day detention time would be satisfactory and that a 10-day detention time would be conservative, but could serve as a working basis for unit design until the process is further evaluated.

Organic particulates entering a unit are mostly consumed in the immediate vicinity of inflow where masses of Daphnia congregate. Organic loading, measured by the BOD_5 test, would not actually be relative as the test is based primarily upon respiratory requirements of fermentative organisms. The significant portion of the oxygen demand in a culture unit would probably be exerted by the Daphnia dominated zooplankton community.

Daphnia survive quite well when only a trace of dissolved oxygen exists. Mixing of nominally clean waters of unit for soluble sulphide suppression would take precedence over aeration per se, although mixing of contents entails aeration. Mixing-aeration should be minimal to needs as Daphnia are weak swimmers unable to resist even slight currents. The single point, fixed feature of mixing-aeration utilized in the Giddings culture unit was excessive, and dissolved oxygen levels fluctuated around 5 ppm.

PRODUCTION - HARVEST - MARKETING

DeWitt and Candland[6] relate that commercial Daphnia harvest in California stabilization ponds commenced in 1968. The amount harvested was 40 tons, with one pond yielding 25 tons at a rate of 1.5 tons per acre per month. Bogatova and Askerov[15], mass culturing Daphnia in concrete tanks on media including yeast and fertilizer, achieved a sustained yield of 76.7 lbs. per acre foot per day. One Texas pond system, consisting of four 35 acre ponds 5 ft. in depth, produces Daphnia 4-5 months each year. Even at a somewhat lower production rate than those above, the harvestable tonnage of Daphnia would be considerable. Daphnia in California were harvested by hand nets and a pump equipped with screening device. A man with a dip net was said to be able to harvest up to 100 lbs. per hour when standing crop was high. A 100 gpm pump was used to pump pond water

Figure 6

NITRATE ACCUMULATION IN CULTURE UNIT 1973

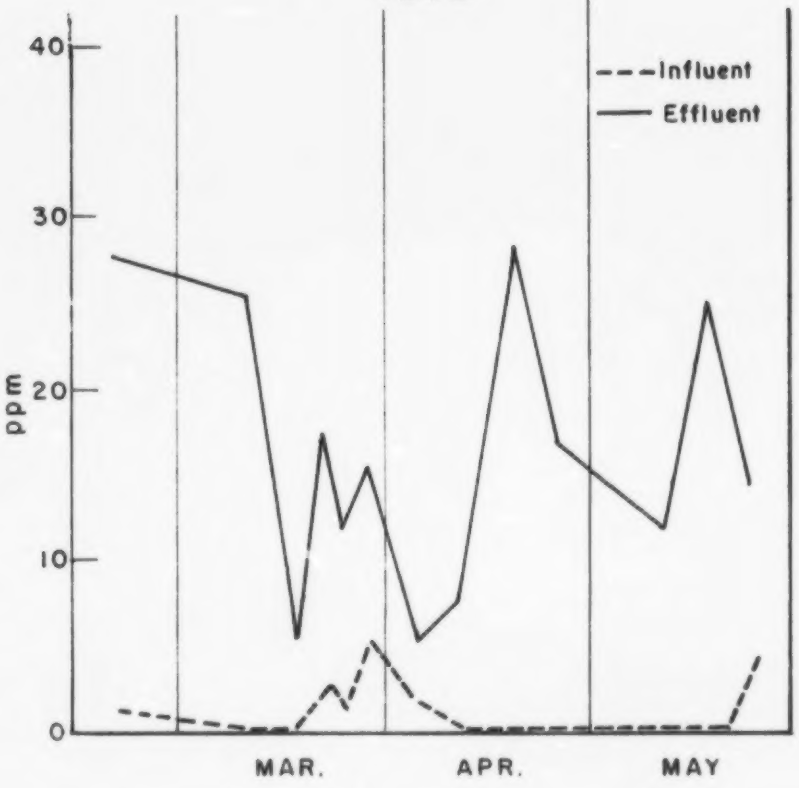
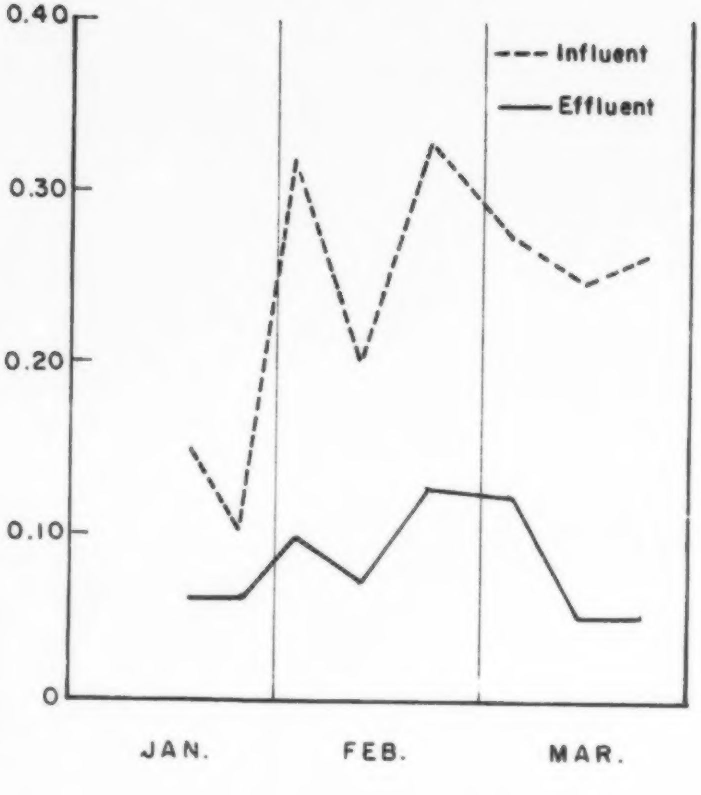


Figure 7

IRON ACCUMULATION IN CULTURE UNIT 1972



onto a screen on one occasion and rate of *Daphnia* harvest attained was 2 lbs. (wet-drained) per minute.

Various types of manufactured screening equipment suitable for *Daphnia* harvest are available as well as low speed separators. Screen mesh sizes of 40-60 would serve to remove most *Daphnia* from water. A proposed approach to *Daphnia* harvest would be to pump culture unit water through removal device with discharge back to unit. Baby *Daphnia* and food material would return to unit and aid in sustaining production. A movable, lightweight, perforated boom supported by floats could serve as a collector for pump input. *Daphnia* tend to swarm and form dense localized concentrations. The boom could be moved to such areas as required.

There is little need to expound about an ever increasing need for protein as animal food additives. Wastewater produced *Daphnia* may represent a significant unexploited potential source of animal protein for this purpose.

Analysis of a sample of *Daphnia pulex* collected from the Giddings culture unit indicated a content of 65.3 per cent protein on a dry weight basis. The potential market for *Daphnia* is probably considerable. The existing market is quite limited. DeWitt and Candland[6] stated that the 40 ton harvest of 1968 in California was sold to wholesalers to be used as aquarium fish food. They report that frozen *Daphnia* brought 15 to 25 cents per pound when sold to wholesalers, depending on market need and size of order. They further pointed out that the aquarium fish food market would soon be flooded should *Daphnia* production become commonplace.

It is believed that one excellent use for *Daphnia* would be as protein supplement in fish food pellets used by hatcheries and commercial fish farmers. Ghadially[16] has pointed out that *Daphnia* are first class fish food, but that an exclusive diet of *Daphnia* provides excessive roughage causing a laxative effect resulting in poor growth. Production of bait fish, such as golden shiner minnows, in a nearby separate pond and fed on harvested *Daphnia* could prove to be feasible. *Daphnia* could also be employed as protein additives in pet and livestock foods.

DISCUSSION

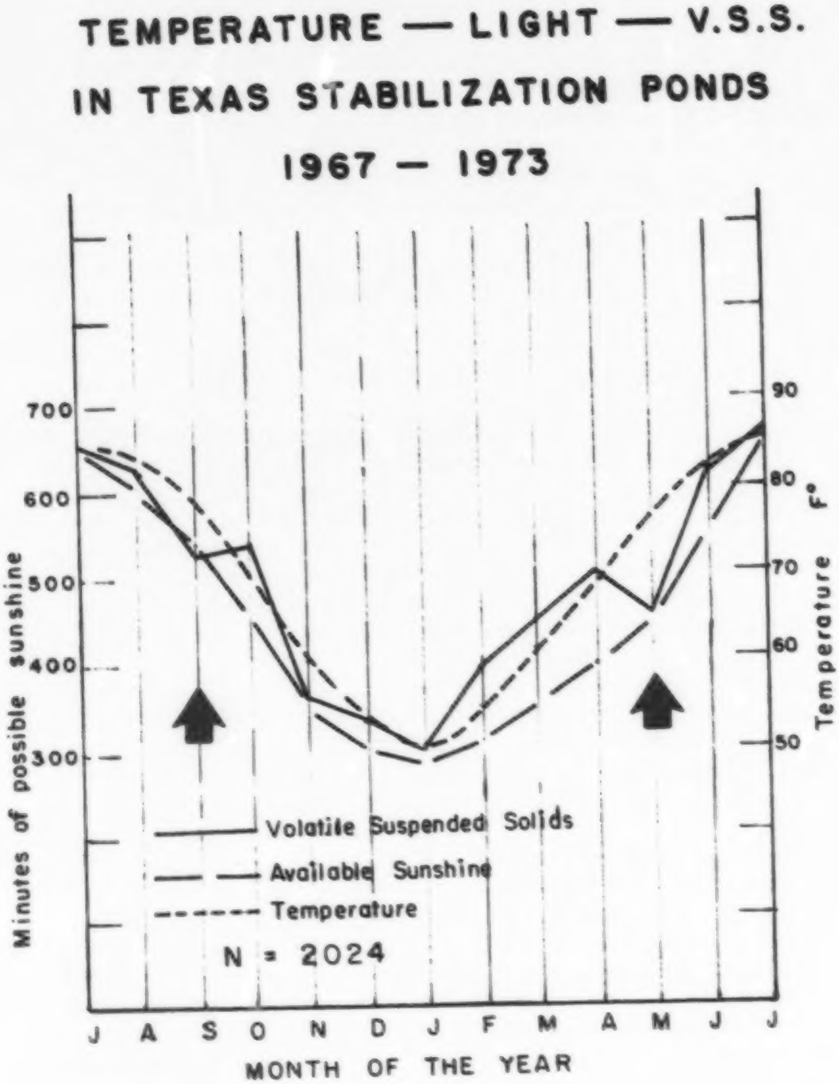
Several thousand stabilization ponds are currently in use in the United States. Some would favor outright abandonment of ponds for wastewater treatment considering that their ultimate efficiency has been attained and found wanting due to excessive production of volatile suspended solids which are largely composed of algae cells. Others feel that this economic, dependable process can be further improved through objective research with close cooperation between engineers and biologists. Figure 8 reveals relationship of light-temperature to volatile solids levels in Texas stabilization ponds as determined from results of analysis of 2,024 effluent samples collected from 1967-1973. Minimum average indicated production is about 40 ppm, with volatile solids level in summer being around 80 ppm. May-September anomalies denoted by arrows could reflect seasonal algae species change. The May anomaly had been noted previously by Dinges[17].

An organism that may have potential value for improving wastewater treatment plant effluent either alone, or in conjunction with Daphnia culture is the introduced asiatic fresh water clam Corbicula. These small clams have a very high reproductive potential and are able to clarify water rapidly. They have been spreading throughout the United States since the 1930's and Dinges[18] has reported that they now infest three Texas river basins. During this study, clams were placed in wire baskets and suspended near the outfall of the Giddings stabilization pond. The animals promptly died. There is a need to define their basic environmental requirements. It may well be that like Daphnia, they are sensitive to ammonia and soluble sulphide toxicity.

CONCLUSIONS

1. Incorporation of advanced biological methods into wastewater treatment systems to make use of fertile effluents for cultivation of beneficial organisms appears feasible.
2. Daphnia culture in wastewater effluent requires pH control within a range of 7.0-7.5 and sufficient mixing for suppression of soluble sulphides.
3. Equipment exists for harvest of Daphnia, but the market is now limited to aquarium fish food. It is believed that a potential market for Daphnia as a protein source for animal foods exists.
4. Advanced biological wastewater treatment offers the possibility of salable products to offset treatment costs, or perhaps to turn a profit. The resulting clarified effluent is conditioned for biological, or chemical-physical methods for further nutrient removal and is still available for irrigation or industrial use.

Figure 8


TEMPERATURE — LIGHT — V.S.S.
IN TEXAS STABILIZATION PONDS
1967 — 1973
700
600
500
400
300
Minutes of possible sunshine
90
80
70
60
50
Temperature F°
Volatile Suspended Solids
Available Sunshine
Temperature
N = 2024
J
A
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O
N
D
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A
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J
MONTH OF THE YEAR

ACKNOWLEDGEMENTS

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REPORT ON PILOT AQUACULTURE SYSTEM USING DOMESTIC WASTEWATERS FOR REARING PACIFIC SALMON SMOLTS

George H. Allen and Larry Dennis[1/]

BACKGROUND TO PROJECT

The utilization of animal wastes, and particularly human wastes, to fertilize waters for improving the growth of aquatic organisms has an ancient past (Allen[1, 2]; Mortimer and Hickling[3]). Most reclaimed wastewaters used for food and fiber production in the United States is for increased plant growth on land (Law[4]; Wilson and Beckett[5]). "Waste treatment lagoons" have grown rapidly in treating a wide variety of wastes, especially treatment of domestic wastes by smaller communities (McKinney, Dornbush and Vennes[6]). Such lagoons are now attracting attention because of their potential for management as highly fertilized fish ponds (Ryther, Dunstan, Tenore and Huguenin[7]). Catfish (Huggins and Backman[8]; Hallock and Zeibell[9]), bait fish (Trimberger[10]) and salmonids (Anon.[11]) have been reared in these lagoons in the United States.

In California about one percent of the state's water demand is supplied by re-use of wastewaters (Deaner[12]). About 85% of the use in California is for agriculture, thus wastewater re-use for production of food and fiber in California is primarily in agriculture (Deaner[13]). Of the 140 reclamation sites listed for 1971, about 100 included some type of lagoon in the system, but no uses involving fish rearing were listed. Reclaimed water was reported in use in four recreational lakes and in four other lakes for miscellaneous purposes. Studies of these systems have been primarily on their reliability of operation in order to protect public health (Jopling, Deaner and Ongerth[14]). The potential uses of algae grown in wastewater ponds in California has had a long history of investigation in California.[6]

In 1955, the City of Arcata constructed a 55-acre oxidation pond on the intertidal flats of the north arm of Humboldt Bay (Figure 1). This was the first stage in protecting commercial oyster beds, and other aquatic resources, from human fecal contamination. Fisheries personnel early became interested in the potential of this oxidation pond for aquaculture, and three studies were directed, either entirely or in part, toward this aim (DeWitt[15]; Hansen[16]; Hazel[17]). Subsequently the City has installed a primary treatment plant, a facultative aeration pond, and a chlorination unit at the oxidation pond outlet (Allen, Conversano and Colwell[18]).

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Initial formal proposals for aquaculture studies in the Arcata system were made in 1963, with financial support available from the Humboldt County Board of Supervisors. A pond system was approved for funding as a demonstration project by the U.S. Public Health Service but never received sufficiently high priority for appropriations. Preliminary studies of the pond water through 2-week standing water bioassay using chinook salmon fry indicated that the undiluted water of the oxidation pond under oxygen saturation was non-toxic during winter months (Allen and O'Brien[19]). When the ratio of sewage to diluting water from infiltration in to the sewer collecting system increased with cessation of winter rains, the oxidation pond effluent became toxic to fingerling salmon by June. These studies indicated the feasibility of a pilot project, which was subsequently funded by the California Department of Fish and Game, Wildlife Conservation Board. Two ponds, each about 1/3 acre (0.15 hectare) in surface area, were placed into operation in July 1971. Fryer[20] recently reported the need to demonstrate in pilot projects preliminary results on vibriosis control, developed in laboratory experiments.

Modern methods for the culture of salmon and trout (Oncorhynchus and Salmo) employ highly mechanized operations (Leitritz[21]). This has resulted because of historically high labor costs in comparison to energy costs. Mechanization has included automatic feeding machines using pellets made in part from high-quality animal protein, particularly marine fish. The cost of both plant and animal protein used in fish foods, along with the cost of energy to run machines, has been sharply rising within the last few years. Thus any simple aquaculture system for rearing trout or salmon utilizing natural food chains developed by low-cost fertilization, or by feeding low-cost raw materials, will have immediate practical application in salmonid culture.

GENERAL OBJECTIVES OF PROJECT

The initial objective of our pilot project was to test by empirical means the capacity of brackish and saltwater fertilized with treated domestic wastewaters to produce food for juvenile salmon. Our investigations have the ultimate practical objective to ascertain if this cultural technique can rear young salmon to migratory size (smolts) as effectively and economically as salmon cultural methods now employed.

Once data have been gathered to meet our primary objectives, the system lends itself to experiments with a wide range of local species of fish and shellfish, both singly, or in various selected combinations (polyculture).

OBJECTIVES OF THIS REPORT

This paper reports the results of six salmon rearing experiments completed from July 1971 through December 1973. Four experiments involved short-term rearing of fingerling coho salmon (O. kisutch) conducted during summer through late-fall periods. Two experiments involved 3-4 month rearing of chinook salmon fry (O. tshawytscha) during winter-spring growing periods. As previously mentioned, for both coho and chinook salmon experiments, the objective was to produce smolts. Smolts are juvenile salmon that lose characteristic lateral marks (parr marks) as freshwater feeding fish, become silvery in color, and physiologically become adjusted for salt-water osmoregulation. Results of these experiments are reported in terms of survival and growth. Factors causing mortalities are discussed, along with evaluation of the results and future plans.

ACKNOWLEDGEMENTS

Funds for the capital cost were provided by the Wildlife Conservation Board, California Department of Fish. Operating funds have been provided through the California State University Coherent Area Sea Grant Program. The continued interest and encouragement of both the administrative and elected officials of the City of Arcata have been a great source of satisfaction as it involves considerable cooperation and goodwill by all parties involved. Mr. Al Merritt, in charge of the recirculating fish hatchery at the University campus, reared most experimental salmon used in the system. He also conducted bioassay in our hatchery of culture waters used in pond experiments. Many undergraduate and graduate students have conducted studies in the system and provided much voluntary help. Many faculty have directed student projects involving aspects of the pond system. To these many people we wish to extend acknowledgement of their aid, and express our sincerest appreciation.

DESCRIPTION AND OPERATION OF SYSTEM

PHYSICAL FEATURES

Fish ponds are located on the west side of the oxidation pond to allow access to a small creek channel located between the oxidation pond and a landfill dump (Figure 1). Saltwater taken from this ditch is introduced into the ponds through a 3'-diameter tar-coated pipe fitted on the pond-side with an Armco cast-iron valve (Headgate, Figure 2). Wastewater is introduced into North Pond by a 1'-diameter pipe located in the northeast corner of North Pond which connects with the oxidation pond. There is no pipe connecting the oxidation pond with South Pond, but fertilization can be accomplished by use of a Barnes trash pump capable of pumping at about 350 gallons per minute. A fish-collecting basin located immediately in front of each headgate is the deepest part of each pond (about 2.0 meters). Each basin also contains a small sump allowing complete dewatering of each pond by use of the trash pump. Headgates are fitted with stainless-steel screens of 1/8-inch diameter circular openings.

Three different types of material were used in construction of the ponds. A yellow sandy loam (Hookton Series) was used for pond banks.

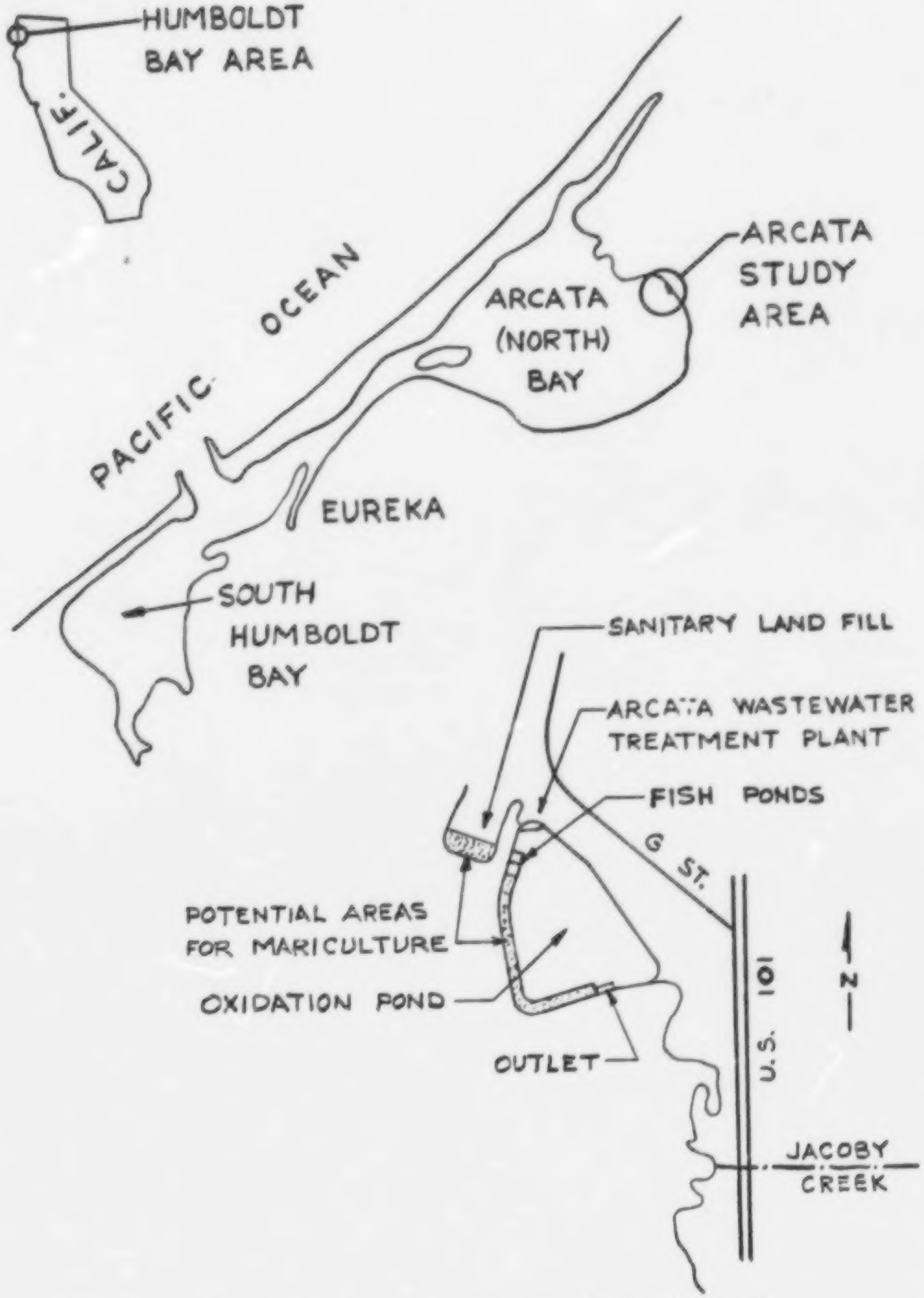
HUMBOLDT BAY AREA
CALIF.
PACIFIC OCEAN
ARCATA (NORTH) BAY
ARCATA STUDY AREA
EUREKA
SOUTH HUMBOLDT BAY
SANITARY LAND FILL
ARCATA WASTEWATER TREATMENT PLANT
FISH PONDS
G ST.
POTENTIAL AREAS FOR MARICULTURE
OXIDATION POND
OUTLET
U.S. 101
N
JACOBY CREEK

Figure 1. Location of study areas mentioned in text.

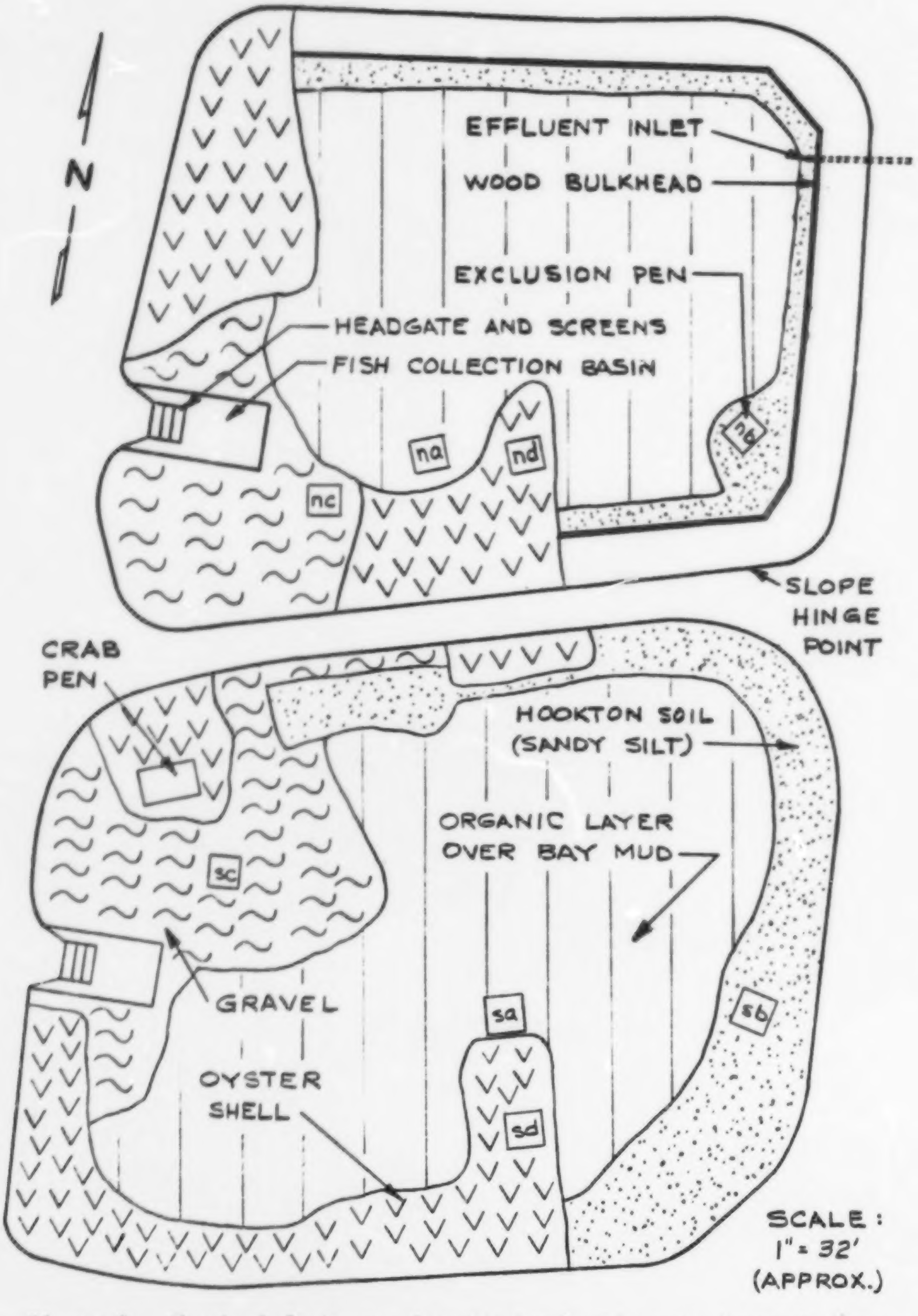
N
EFFLUENT INLET
WOOD BULKHEAD
EXCLUSION PEN
HEADGATE AND SCREENS
FISH COLLECTION BASIN
na
nd
nb
nc
SLOPE HINGE POINT
CRAB PEN
HOOKTON SOIL (SANDY SILT)
ORGANIC LAYER OVER BAY MUD
sc
GRAVEL
sa
sb
OYSTER SHELL
sd
SCALE: 1" = 32' (APPROX.)

Figure 2. Physical features of North (top) and South (bottom) fish ponds.

River-run gravel was cast around each headgate to allow light machinery access to the area for excavating of fish-collecting basins. A third material, oyster shell, was specifically employed to provide artificial substrate for production of food organisms. This material was placed in several low spots in the pond bottoms and along portions of the pond banks. A fourth material (mud) was the original intertidal sediment plus overlying organic matter deposited during operation of the oxidation pond. Mud covered a majority of the pond bottoms. Pens two-meters square of nylon bobinetting were constructed in each pond over a sample of each of the four substrates. These pens (Exclusion Pens, Figure 2) were originally used to exclude fish from these substrates, but now serve to rear known numbers of fish over each bottom type. The ponds have been operated as static systems to conform to directions from the North Coast California Regional Water Quality Control Board (no waters containing unchlorinated effluents may be discharged into Humboldt Bay without at least two weeks retention time). In practice, we have rarely discharged unchlorinated wastewater that has not had about a month retention in the system. Ponds could be operated as a tidal-flushing system but due to the expense and complexity of having to chlorinate these flows, we have not as yet operated with tidally-flushed ponds receiving a continuous addition of wastewaters.

BIOASSAYS

In order to assist in identifying periods of mortality, or to identify causes of mortality, lots of salmon taken from those planted into the ponds were reared in aquaria in the hatchery (Hatchery Bioassay) or in floating pens in the fish ponds (Live-car Bioassay).

HATCHERY BIOASSAY

Standing-water bioassays were conducted in 20-gallon aquaria kept at hatchery water temperatures by placing the aquaria in hatchery troughs. The aquaria were serviced with compressed air introduced through air stones to maintain oxygen saturation in the water. There was no feeding of lots of salmon being tested in these bioassay experiments.

Hatchery bioassay was undertaken to measure mortalities due to toxicity of water used in salmon culture. Various combinations of oxidation pond water, saltwater from Humboldt Bay or from the open ocean at Trinidad Head, as well as the mixtures of oxidation pond waters and Humboldt Bay water employed in North and South ponds, were tested. Although not designed for the purpose of measuring improvement of water quality from operation of the system, data from such hatchery bioassay can be used to estimate how well Arcata plant effluent meets recently adopted state standards on toxicity (Calif. State Water Resources Control Board[22]). Only a small part of hatchery bioassay data are reported here.

LIVE-CAR BIOASSAY

Floating pens (live-cars) were employed to obtain daily counts of mortality of salmon. Two types of cages were employed (Table 1). One type (cage) was made from 2" x 4" wooden frames fitted with nylon bobinetting. A large cage 4' x 4' x 2' employed mesh of hexagonal openings 7 mm in longest and 3 mm in shortest diameter. A small cage 2' x 2' x 2' employed circular mesh of 1 mm diameter. A second type of live-car used was called an "insert" because of its mode of construction. A 2' square wooden frame was fitted with plastic floats and a hinged lid. An inside lip was made to hold a frame which was used to support a collapsible hanging net of mesh 7 mm by 3 mm hexagonal openings. The lower portion of the net was kept vertical and square by sewing into the lower perimeter a stainless steel square frame.

Accurate daily mortality counts using cages were not available. Due to the size and weight of the cages it was difficult for a single man to entirely remove the cage from the pond. Dead salmon could remain undetected on the bottom of the cage where a carcass could be completely consumed by amphipods in less than 12 hours when high numbers of amphipods were present in the ponds. The insert live-car could be easily removed from the pond by one man, thus greatly increasing reliability of daily mortality measures with this type of live-car.

AERATION AND MIXING

Upwelling

Initially we used a low-cost system producing "upwelled" water (Figure 3). Water was taken from the surface by a small electrically-driven water pump and discharged through a simple manifold at the bottom of each fish-catching basin. The intake jet was kept on the surface by a styrofoam plastic float. The systems created an "upwelling" immediately adjacent to each headgate. Whenever aquatic higher plants occurred in the system, constant plugging of intake and impellers in the pumps developed. The pumps were not marine models and deteriorated rapidly in the wastewater-saltwater solution.

Spray

A spray system was the second method of aeration employed (AIR-O-LATOR, Roycraft Industries). The instruemnt drives surface water into the air by use of a small impeller rotating at very high speeds (Figure 4). As originally mounted by the manufacturer, the instrument did not provide protection for salmon against the impeller blades. We mounted the instrument on a styrofoam block, fitted into the end of a 55-gallon oil drum. The lower end of the drum was covered with nylon-mesh to exclude fish. The water enters the drum near the bottom of the pond, moves vertically, to be sprayed into the air. These units have been located near the fish collecting basins. AIR-O-LATOR parts used by us were primarily fashioned for freshwater operations, thus galvanic actions due to saltwater caused complete disintegration of some parts of

Table 1. Description of "live-cars" used in monitoring water quality of pond waters for juvenile salmon and for daily measure of mortality

Live-Car	Description	
	Frame	Mesh
Large Cage	4' x 4' x 2'	Nylon bobinetting; hexagonal mesh; 7 mm long and 3 mm shortest diameter.
Small Cage	2' x 2' x 2'	Nylon bobinetting; circular mesh; 1 mm in diameter.
Insert	2' x 2' with plastic floats	As in large live-car; mesh on removable frame hung 3' deep with stainless steel frame at bottom to maintain shape.
Fry	11" x 14" x 20"	Wire mesh of 1/8" square openings.

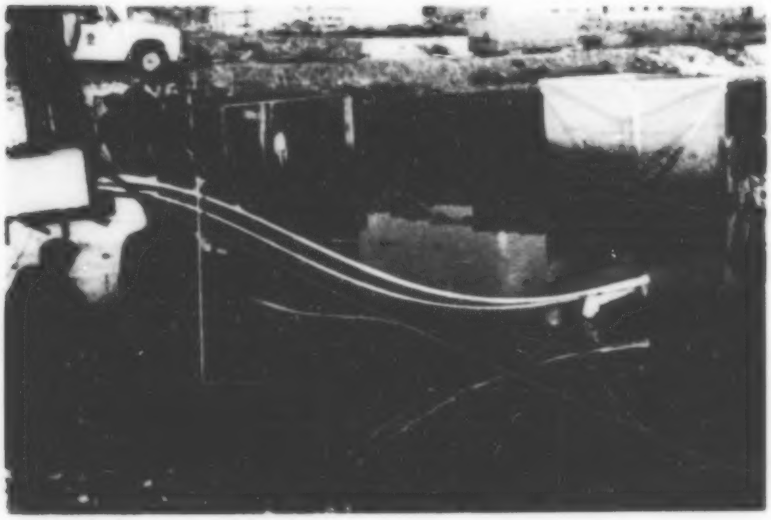

Figure 3. Upwelling mixing and aeration unit, South Pond headgate. Pen (SC) over gravel shown right corner; crab pen middle background.

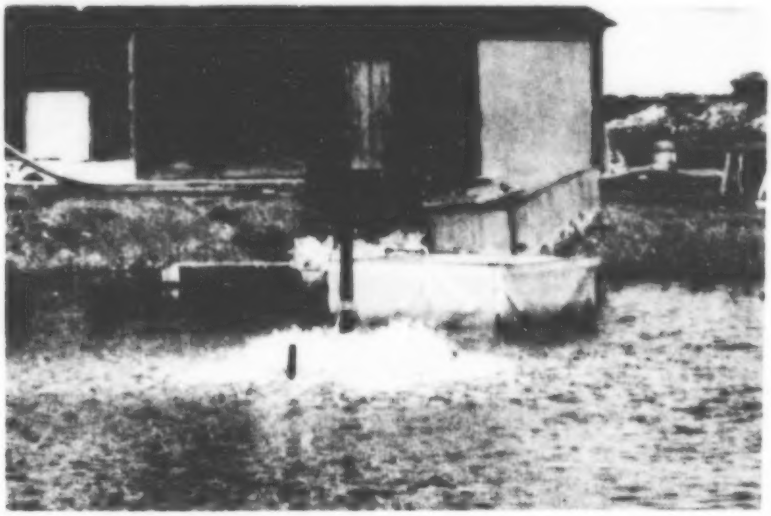

Figure 4. AIR-O-LATOR spray system, South Pond, with Pen (SC) over gravel. Fish barn in background to house recirculating system for holding and marking salmon.

the unit. The electric motor unit (Franklin), however, appears quite rugged and watertight, and has performed adequately. Plastic parts have now become available and are to be tested in the system.

Air

North Pond was fitted with a forced-air aeration system during the summer of 1973 (Figure 5). Air lines with associated jets are located immediately off the portion of the North Pond bank not associated with an oyster shell covering. A vertical 2-foot wall exists in this portion of the pond, and the air jets breaking the water surface plus the vertical bank serve to discourage predation by fish-eating birds (herons, egrets, night herons). A second pipe leads directly to the fish catching basin to a U-shaped manifold insuring complete mixing of all pond waters.

POND WATER PROPERTIES

Daily observations of water temperature, turbidity, hydrogen ion concentration, dissolved oxygen, and salinity were made from surface samples obtained at the headgate of each pond during morning hours (8-10 AM). Additional water properties were studied only intermittently.

Considerable day-to-day fluctuations in some of the properties occurred (pH, turbidity, oxygen) (Allen[23]). It was possible to fit curves to the raw data by eye showing major trends during each experiment (Figures 6-9). Time of each experiment is listed in Table 2.

Water temperature was primarily controlled by air temperature. Local weather conditions (storms in winter, cloud or fog cover in summer) cause yearly variations. During summer months, the ponds ranged near 20 C (Figure 6). Such temperatures are found in estuaries and lagoons in northern California and Oregon at this time of the year (Joseph[24], Hayes[25], Reimers[26]). Ice covered our ponds once during the two winters of operation to date and then only for a day. Diurnal changes in temperature, as determined from cursory analysis of thermograph charts obtained from an instrument operated about a foot under the water surface, are relatively small (2-3 degrees at most).

Turbidity, primarily a function of phytoplankton populations, showed high variability during coho rearing periods (fall and early winter) (Experiments I, III, IV, VI) (Figure 7). High turbidity from phytoplankton occurred during both chinook rearing periods (winter-spring) (Experiments II, V).

Hydrogen ion, primarily a function of photosynthesis, was always quite high, generally between values of 8 to 9.5, with a few cases of values near 10 (Figure 8). No similarity in trends could be discerned during winter periods (Experiments I, III, IV, VI) although the average value was about pH-8. In contrast, the winter-spring conditions produced pH's tending toward values in late spring of 9-9.5.

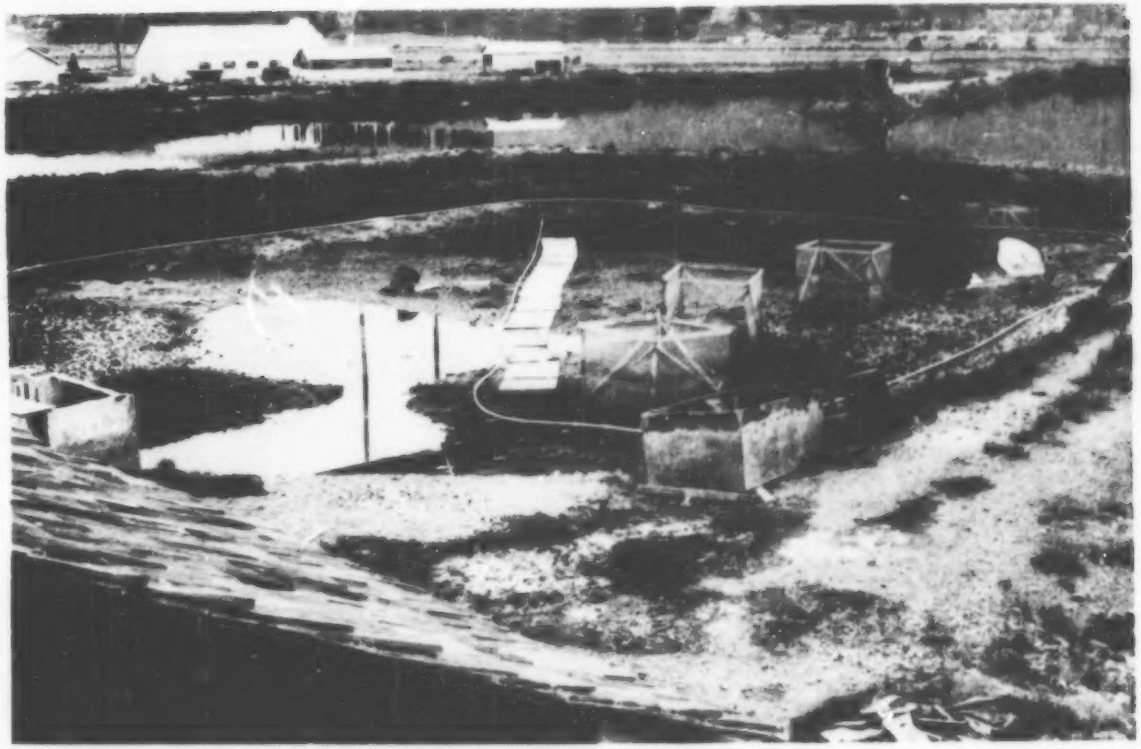

Figure 5. North Pond during pond modification, summer 1973, showing retaining wall and air line at base.

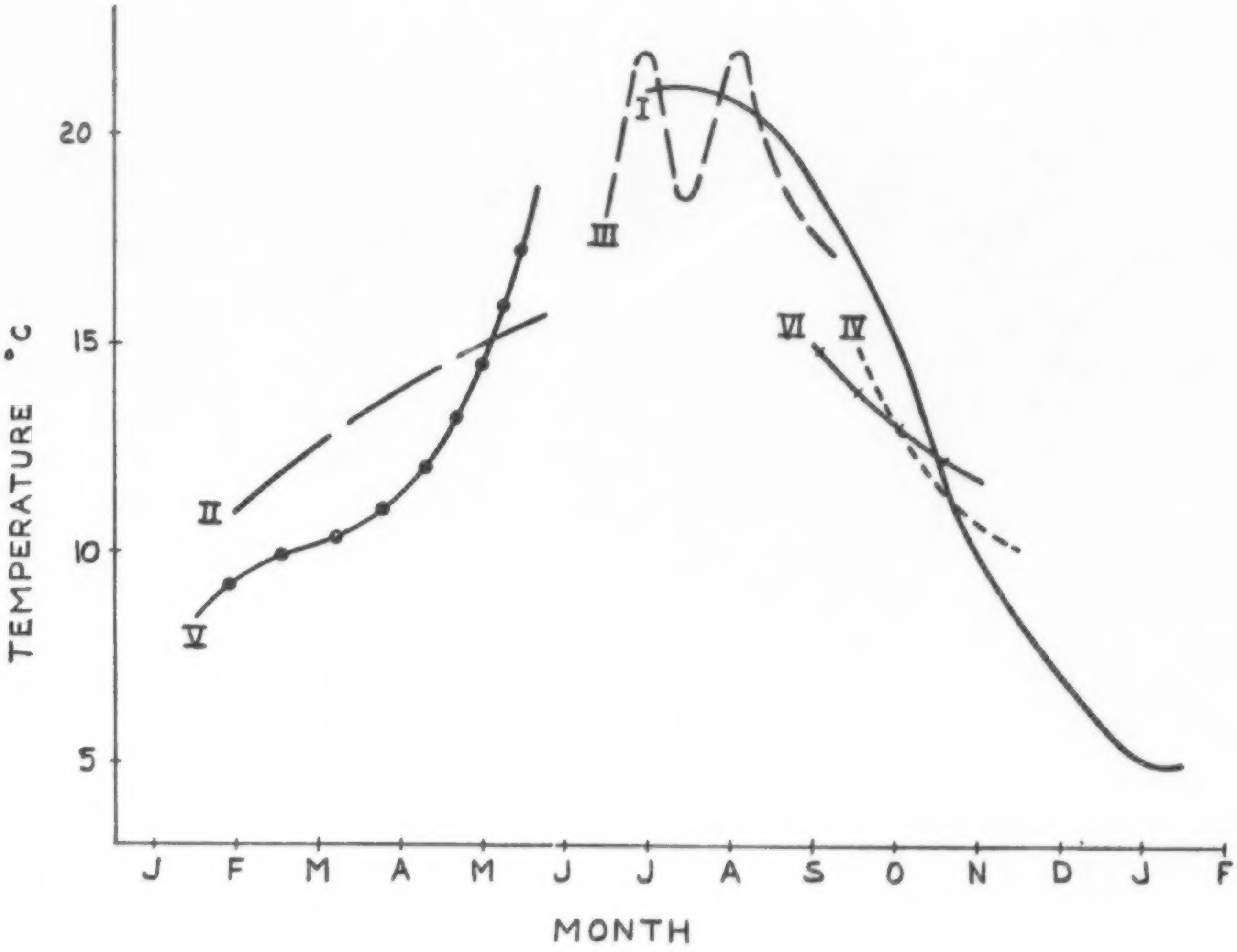

Figure 6. Trends in pond water temperatures, North Pond, salmon rearing

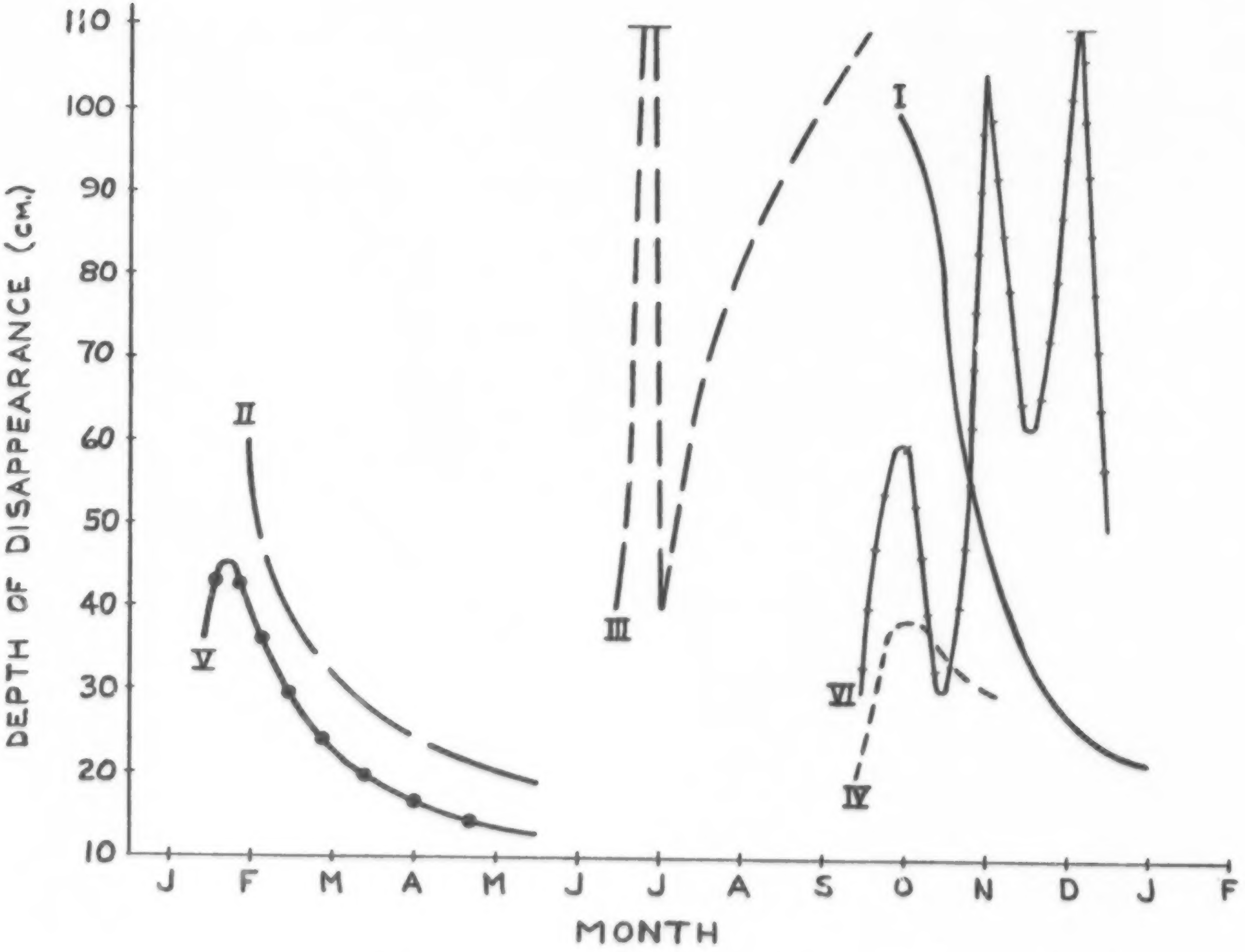

Figure 7. Trends in water clarity, North Pond, salmon rearing experiments I-VI.

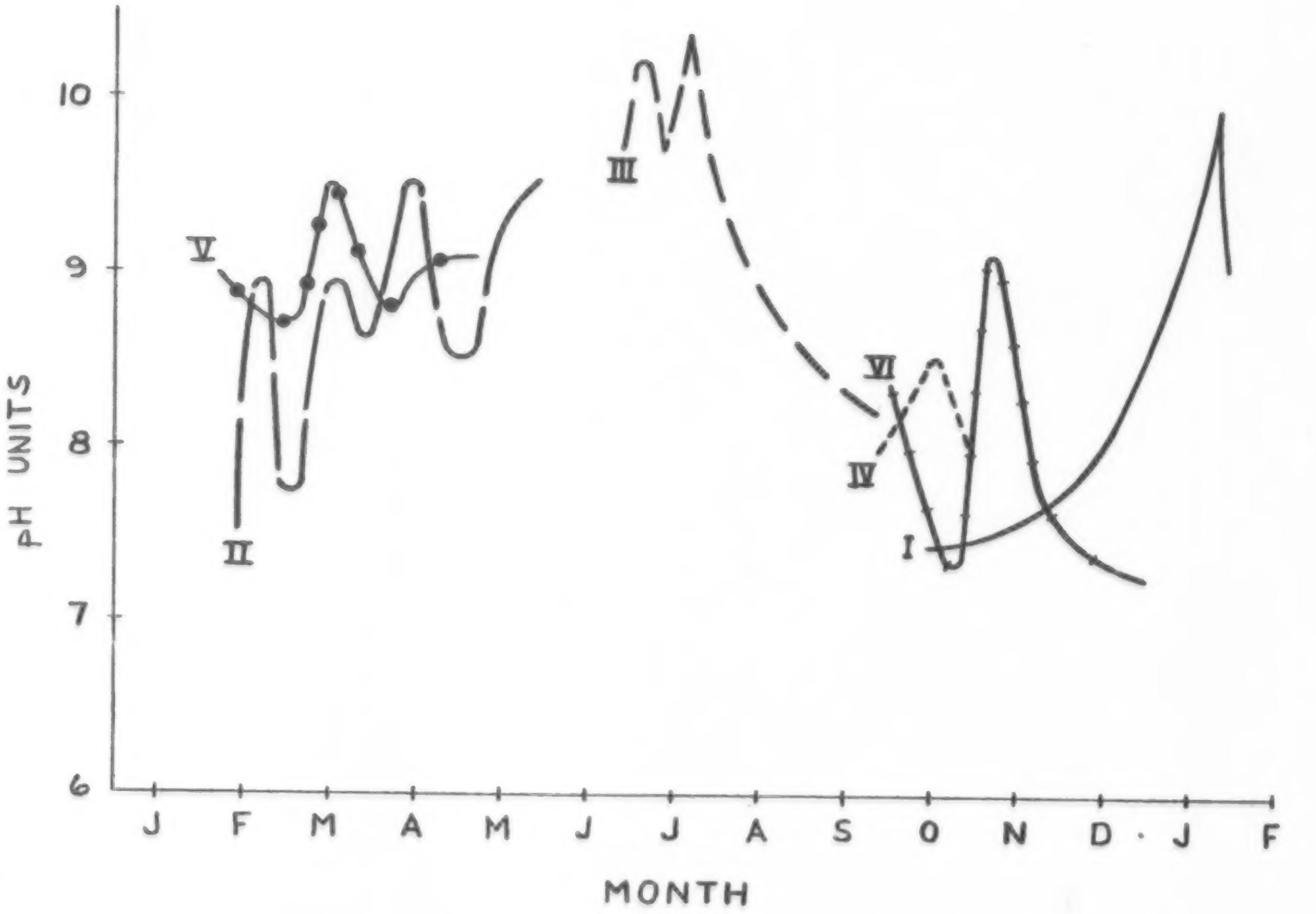

Figure 8. Trends in hydrogen ion concentrations, North Pond, salmon rearing experiments I-VI.

Dissolved oxygen was always high, and only on a few occasions fell below 5 mg/l (Figure 9). In those instances, salmon mortalities could be correlated with these low values, especially during periods when water temperatures were high. No pattern was discerned in oxygen trends during the fall-winter experiments (Experiment I, III, IV), except for Experiment VI which under Air aeration maintained a constantly high oxygen level. Winter-spring patterns were somewhat similar with high initial levels followed by a slowly dropping trend, and a sharp drop at the latter portions of the experiment (Experiment II and V). These low points are associated with periods of intense overcast from local heavy fogs that have followed a period of bright sunshine.

Salinities have been the most stable property, with any sharp changes occurring toward lower salinity values as associated with periods of heavy rainfall. Adjustments in pond salinities were made to experimental values by small discharges of pond water to the bay and addition of higher-salinity water in return. During spring periods, salinities have slowly tended to increase, probably due to evaporation. Salinity ranges have not been graphed but are listed with tabular data as needed.

DESCRIPTION OF EXPERIMENTS AND SALMON SURVIVAL

Salmon rearing has been grouped into six periods (experiments) (Table 2). Eleven lots of salmon were planted as part of these experiments. To date, coho salmon have been planted only as fingerlings (juvenile salmon 6-9 months old). Coho rearing has been conducted during the summer (Experiment IA; Experiment III), or during late fall, (Experiment IVA; Experiment IB, IVB, and VI). Chinook salmon experiments employed fry (young salmon that have absorbed their yolk-sac and have been actively feeding from two-four weeks). Chinook rearing has taken place only in winter (Experiment II and V).

Salmon utilized in our experiments were obtained from the California Department of Fish and Game hatcheries located on Mad River, Humboldt County, California, and on the upper Klamath River (Iron Gate fish hatchery). Salmon planted into the ponds were mainly reared at the Humboldt State University recirculating fish hatchery although some were reared at the Mad River hatchery or a Humboldt County hatchery located on Prairie Creek.

EXPERIMENT IA

Water was initially introduced into North Pond on July 21, 1971 and it was allowed to mix until July 23 when initial plants of coho salmon were attempted. From July 23-28, 5,000 fingerling coho salmon were introduced into each pond. Water quality in North Pond was poor and salmon with darkened coloration and listless behavior were observed on the surface after about three days residence. In South Pond, coho planted had been acclimatized for 48 hours at 15 o/oo salinity at 16-17°C temperature, but this was unsuccessful as acute mortalities occurred almost immediately in salinities of 32-32.5 o/oo (parts per thousand).

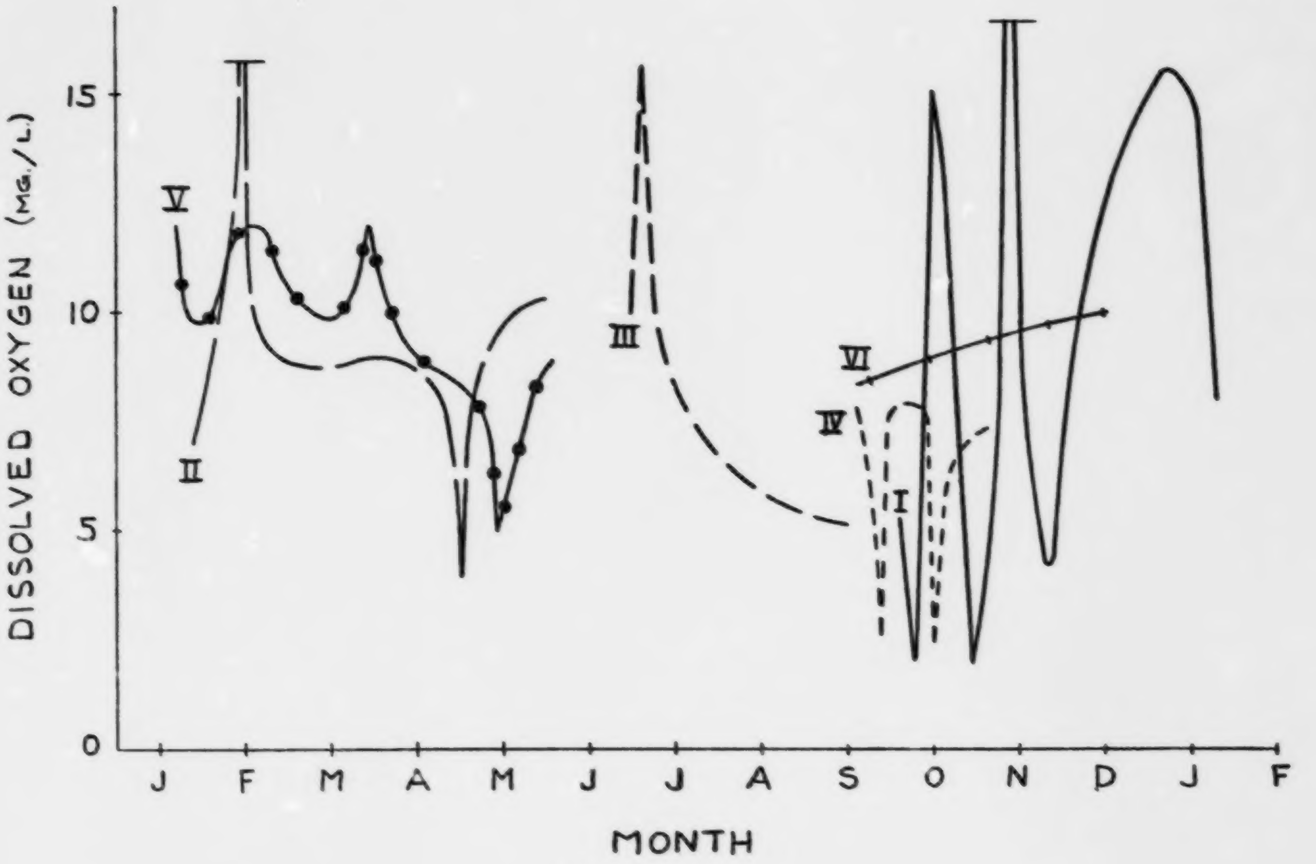

Figure 9. Trends in oxygen concentration, North Pond, salmon rearing experiments I-VI.

Table 2. Summary of rearing experiments in North Pond with juvenile Pacific salmon using Humboldt Bay seawater mixed with wastewater from City of Arcata oxidation pond.

Experiment No.	Rearing Period	Species	Size of Fish	Days of Rearing	Aeration and Mixing System	Salinity (parts per thousand)
IA	Jul - Oct 71	Coho	Fingerling	-	Upwelling	19-24
IB	7 Dec 71 - 22 Jan 72	Coho	Fingerling	45	Upwelling	13-16
II	11 Feb - 2 Jun 72	Chinook	Fry	111	Upwelling and Spray	11-12
	17 Apr - 2 Jun 72	Chinook	Fry	45	Upwelling and Spray	11-12
III	7 Aug - 22 Sept 72	Coho	Fingerling	45	Spray	16-23
IVA	Oct 72	Coho	Fingerling	-	Spray	13-16
IVB	3 Nov - 17 Nov 72	Coho	Fingerling	14	Spray	15-17
V	26 Jan - 26 May 72	Chinook	Fry	119	Spray	10-13[1/]
	16 Mar - 26 May 73	Chinook	Fry	70	Spray	10-13[1/]
	26 Apr - 26 May 73	Chinook	Fry	52	Spray	10-13[1/]
VI	2 Nov - 15/16 Dec 73	Coho	Fingerling	43	Air[2/]	10-18

[1/] South Pond: 18-20 and spray aeration.

[2/] South Pond: Spray.

The number of salmon recovered from around the edges of the ponds was about seven times as great from South Pond as was recovered from North Pond. North Pond fish tended to sink to the bottom, while South Pond fish skittered on the surface and tended to float. Feeding salmon were observed on South Pond surface but rarely on North Pond. Some coho salmon survived in South Pond until early September. Additional small numbers of coho salmon were introduced into North Pond during August but these all succumbed as indicated by total mortalities recorded in live-car bioassay. Bioassay in live-car continued to show high mortalities through late September.

During July-September 1971, salinities in North Pond ranged between 19-24 o/oo. Water temperatures ranged around 20^{o}C. Oxygen levels showed typical wide diurnal variations associated with highly eutropic conditions. Salmon mortalities in live-car bioassay were correlated with dissolved oxygen level of from 2-3 mg/l.

EXPERIMENT IB

Beginning in mid-October, 1971, coho salmon planted into live-cars in North Pond showed about 70 percent survival over a two-week period. During November, survival increased to about 80 percent of the salmon retained in live-cars over a month's time. Based on these results, 17,000 coho salmon averaging 10.5 grams per fish, were introduced into North Pond, between October 18 and 7 December 1973 (15,000 were planted 7 December 1973). On January 22, 1972, 9.500 salmon were recovered and released into Humboldt Bay. The average weight of the surviving salmon was 15.0 grams. Survival was 55 percent over the 45-day rearing period for the bulk of this fish planted (7 December 1973).

EXPERIMENT II

North Pond remained empty from 22 January until February 9, 1972, when effluent and saltwater were mixed into the pond. Some additional water was added on February 10 and 11. Salinities on 11 February varied from 11 o/oo at the surface to 15.5 o/oo at 3-foot depth. The pond was initially aerated by upwelling, then by spray. The AIR-O-LATOR unit failed during the experiment and was replaced by the upwelling pump refitted for aerial spray. In late April the AIR-O-LATOR was replaced with a new unit, and the pump refitted for upwelling. On 2 June the pond was drained.

Between February 11-14, 1972, 10,000 chinook fry were planted into North Pond, followed by 2,500 fry marked by excision of the left ventral fin (LV). On 2 June, 2.5 percent and 4.0 percent of these two groups of salmon were recovered as smolts.

EXPERIMENT III

On June 28, 1972 a mixture of saltwater and oxidation pond water was introduced into North Pond to form a salinity of 16 o/oo. Live-car bioassay showed high mortalities of coho salmon through July but with slowly improving survival rates throughout the month. During live-car rearing periods temperatures fluctuated around 20° ± 2°C. From July 28 to August 11, three introductions of fingerling coho of about 1,000 fish each were made into water of about 18 o/oo salinity. On 22 September the pond was drained and 3.9 percent of the 3,000 salmon recovered. In addition over 50,000 fingerling topsmelt and sticklebacks were recovered. These latter fish had developed entirely from eggs and larvae in the saltwater introduced from Humboldt Bay at the initial filling of the ponds.

EXPERIMENT IVA

North Pond was filled with seawater and effluent in late September, 1972 and adjusted to produce a salinity of 15 o/oo. Live-car bioassay was begun on 4 October, with no mortality over a 10-day period. In order to repeat Experiment I with a longer period of rearing, 16,000 coho fingerlings were placed into the pond on October 13. A group of 50 salmon was placed into the live-car. On October 13, 19 salmon died in the live-car (38 percent), which correlated with a period of low oxygen level (<3 mg/l). On November 3, only 12 percent of the live-car fish remained. High numbers of distressed fish were being eaten by birds (Blankinship[27]). We assumed that most fish in this experiment had died.

EXPERIMENT IVB

As part of the objective of Experiment IVA was to allow a study of bird predation, we had retained 5,000 fingerlings in our hatchery as reserve, and these were placed into the pond on November 3. On 17 November the pond was drained and 4,220 fingerlings were recovered (84 percent survival over 14 days of rearing). No mortalities occurred in live-car bioassay during the rearing period.

EXPERIMENT V

Both North and South Ponds were filled with seawater during December 1972 but had to be emptied in early January 1973 for some pond repair and maintenance work. Oxidation pond effluent was introduced in mid-January to obtain two levels of salinity (about 9 o/oo for North Pond and 18 o/oo in South Pond). Two stocks of chinook salmon were made available for this experiment. One stock was from early-run salmon returning to Mad River hatchery, and the second stock later-running salmon to Iron Gate fish hatchery on the Klamath River. A group of late-run salmon was retained in the Humboldt State University hatchery for planting in late April in order to allow the salmon to become large enough to be marked by fin excision (left ventral fin - LV). South Pond was drained on 25 May and North Pond on 26 May 1973. Survival of fish over the three rearing periods ranged from 0.1 to 6 percent (Table 3).

Table 3. Survival of chinook salmon fry planted into North and South Ponds, Experiment V.

	North Pond			South Pond		
Stock	Early Run	Late Run		Early Run	Late Run	
Mark	None	None	LV	None	None	LV
Date Salmon Planted	26 Jan	16 Mar	26 Apr	26 Jan	16 Mar	26 Apr
Number Planted	1,800	7,500	2,500	1,800	7,500	2,500
Days Reared	119	70	30	110	70	30
Percent Survival	6	1	1	2	0.1	0.2

EXPERIMENT VI

No studies were conducted during the summer of 1973 due to major modifications and repairs undertaken for the system. A 2-foot vertical wall of redwood planks was constructed along all banks of North Pond except in those areas where the bank was not protected by an oyster shell layer (Figure 5). A forced-air aeration system was installed during this period as previously described. Water from Humboldt Bay was introduced into both ponds during late August. After draining North Pond several times to adjust the aeration system, water from the oxidation pond was added to both ponds to develop a salinity of about 25 o/oo. This was to test Power's[30] hypothesis that amphipod growth and survival would be maximized at this salinity level. In early October, salinities were reduced to about 15 o/oo. On October 4, live-car bioassays were started, with no mortalities recorded through October. On 2 November 1973, 5,000 coho fingerlings were introduced into both ponds. After 43 days rearing, over 80% of the fingerlings were recovered for release into Humboldt Bay. Very few of the salmon had become smolts. Because there were no residual stocks of amphipods left within the pond following summer construction, nor were there amphipods occurring naturally in Humboldt Bay during introduction of saltwater, very few of these forms were available as food for the salmon. Consequently, larger salmon actually showed a slight reduction in weight although smaller fish retained or even slightly improved their condition during the rearing period.

SALMON PRODUCTION

POND REARED FISH

The production of salmon from the system varied widely (Table 4). For coho fingerling planted in late summer and fall periods (Experiment IB, IVB, VI) the trend has been from moderate to high survivals, coupled with an inverse trend in actual fish production (high to low). The single experiment with coho fingerlings reared during a summer period (Experiment III) showed a low survival and low production of salmon but a high survival and high production of other species of fish. Both survival and production of chinook salmon from fry plantings were low, although the chinook grew fast and smolted (Experiment II and V).

PEN AND LIVE-CAR REARED FISH

The survival and growth of salmon reared in pens and live-cars was often markedly different, usually greater, than salmon living unrestricted in the ponds, indicating a potential growth and survival greater than actually found for the ponds to date.

Pen

Pens constructed over pond substrates were designed to exclude fish over these substrates. During Experiment IB, on October 20, 1971, in error 50 coho salmon were placed into Exclusion Pen NC (gravel substrate, Figure 2). Electric shocking, and seining, recovered 35 salmon. When the pond was drained on 22 January 1972, 17 fish were recovered.

Table 4. Summary of production[1/] in fish-rearing experiments, June 1971 - December 1973, in pilot-fish pond system using saltwater fertilized with advanced secondary treated effluent from City of Arcata oxidation pond.

Exp. No.	Pond	Species, Size of Fish	Days Reared	Percent Survival	Production[1/] as kilograms per hectare per day	Remarks on experimental result
IA	North	Coho fingerling	-	0	-	Unstable water and bottom conditions; temperatures consistently over 20 C
	South	Coho fingerling	-	0	-	Salinities over 32 o/oo and high temperatures; high density of topsmelt and stickleback
IB	North	Coho fingerling	45	55	6.8	High density of planting induced intensive predation by birds
II	North	Chinook fry	45-111	3	0.05-0.07	Mortality possibly due to over-rearing.
III	North	Coho fingerling	45	4	0.4	Nature of mortality undetermined. Food primarily consumed by other species

Table 4. (Continued)

Exp. No.	Pond	Species Size of Fish	Days Reared	Percent Survival	Production[1] as kilograms per hectare per day	Remarks on experimental result
	North	Topsmelt, stickleback, sculpins	86	high	4.2	Production entirely from survival of eggs and larvae introduced with saltwater from Humboldt Bay
IVA	North	Coho fingerling	-	0	-	Mortalities correlated with period of low dissolved oxygen
IVB	North	Coho fingerling	14	84	3.7	Experiment primarily to assist in bird predation study
V	North	Chinook fry	30-119	1-6	trace	Mortality possibly from over-rearing
	South	Chinook fry	30-119	0.1-2	trace	Mortality possibly from over-rearing
VI	North	Coho fingerling	43	96	loss	Sufficient amphipod population did not develop in system
	South	Coho fingerling	43	78	loss	" "

[1] Production defined as the change in weight of those fish surviving from planting into ponds until recovered during pond draining.

Table 5. Comparison of survival of chinook fry planted into ponds and reared in exclusion pens (Experiment V).

	North Pond		South Pond	
	Exclusion Pen Over Gravel	Pond	Exclusion Pen Over Gravel	Pond
Number Planted	77	7,500	77	7,500
Number Recovered	40	85	14	12
Percentage	51	1	18	0.1
Surface Area (Square meters)	4	1,500	4	1,500

Table 6. Comparison of survival of coho fingerling salmon planted into ponds and reared in exclusion pens (Experiment VI).

	North Pond		South Pond	
	Exclusion Pens	Pond	Exclusion Pens	Pond
Number Planted1/	56	5,000	56	5,000
Number Recovered	462/	4,800	56	3,900
Percentage	82	96	100	78

1/ 7 unmarked and 7 LV marked coho fingerlings planted into each of four pens.

2/ 8 of 10 mortalities from pen NB (Hookton Soil).

This represented virtually no mortality during a 92-day rearing period. At the same time, live-car reared fish showed 20-30 percent mortalities, compared to a 45 percent mortality suffered by the salmon released into the pond.

Based on this experience, we used the pens to study the effect of having different densities of salmon feeding over the substrates in Experiment V. Our examination of the pen bottoms failed to disclose small escape route for small fish under the corners of the pen frames except for the pens over gravel. Consequently salmon escaped through these holes during pond draining, and an absolute count of salmon surviving was made from the pens over gravel in each pond (Table 5). In both ponds, survival of chinook fry in the pens was strikingly higher than the generally low survival of the same lot of fish planted into the ponds.

During the summer of 1973, each pen bottom was made secure. At this time pen NB was relocated over fresh Hookton soil. We then planted each pen at the same density of salmon per unit area as we did in the main ponds (Experiment VI). All salmon survived in South Pond pens, while mortalities in North Pond pens were mainly associated with fish grown over raw Hookton Soil (Table 6). High survival in general made it impossible to draw any conclusions on substrate effect. The new Hookton soil probably did not provide sufficient food for the salmon.

Live-Car

For coho fingerling salmon, the confinement of salmon in pens suspended away from the pond bottom resulted in less growth than salmon reared in cages located on pond substrates. In Experiment VI, both LV and unmarked lots of salmon were consistently smaller when grown in cages than when reared in pens over bottom substrates (Table 7). This effect was first noted in Experiment II when the average size of the salmon in North Pond was 12.4 grams in pen located over gravel in comparison to 15.0 grams for the salmon reared in the pond. It was not possible to discern whether one substrate was more beneficial over another in Experiment VI because individual fish were not measured for weight or length before being placed into pens.

In the single experiment with chinook fry lots reared in cages and pens (Experiment V), the rate of growth was slower in cages than in the pond but the actual production of wet weight of salmon was higher in the cages (Table 8). These production rates were in the range exhibited by coho fingerling in experiments IB and IVB (Table 4).

CAUSES OF MORTALITY

OXYGEN DEPLETION

The use of oxidation pond effluent to fertilize seawater produces

Table 7. Comparison of average weight in grams of small and large-sized coho fingerling salmon reared in cages and pens (Experiment VI).

Pond	Rearing Site	Period	Days	Weight by group of fingerling	
				Small (LV)	Large (Unmarked)
North	Insert and Culture Raft	4 Oct - 15 Dec 73[1]	73	6.3 (24)[2]	-
	Insert Live-Car	2 Nov - 15 Dec 73	44	5.3 (23)	17.6 (26)
	Shell Pen	2 Nov - 15 Dec 73	44	7.6 (5)	27.1 (7)
	Hookton Soil Pen	2 Nov - 15 Dec 73	44	9.0 (5)	1.0 (1)
	Gravel Pen	2 Nov - 15 Dec 73	44	8.0 (7)	18.0 (7)
	Mud Pen	2 Nov - 15 Dec 73	44	7.5 (7)	18.6 (7)
South	Insert and Culture Raft	4 Oct - 16 Dec 73[1]	74	4.2 (24)	
	Insert Live-Car	2 Nov - 16 Dec 74	45	4.8 (30)	13.8 (30)
	Shell Pen	2 Nov - 16 Dec 74	45	8.0 (7)	17.2 (7)
	Hookton Soil Pen	2 Nov - 16 Dec 74	45	6.8 (7)	15.9 (7)
	Gravel Pen	2 Nov - 16 Dec 74	45	9.5 (7)	19.1 (7)
	Mud Pen	2 Nov - 16 Dec 74	45	8.5 (7)	19.6 (7)

Table 7. (Continued)

Pond	Rearing Site	Period	Days	Weight by group of fingerling Small (LV)	 Large (Unmarked)
South	Crab Rearing Pen[3/]	2 Nov - 16 Dec 74	45	6.6 (21	18.0 (2)

[1/] Small salmon without a mark were placed in Insert live-car on 4 October, reared until 2 November, then moved to hanging pen in Culture Raft.

[2/] Number of salmon recovered at end of rearing period shown in parenthesis. Twenty-five salmon were placed in Insert live-car. Seven salmon each of small and large-sizes were placed into pens.

[3/] Salmon entered pen on their own and were recovered on draining pond.

Table 8. Comparison of rates of production of wet weight of salmon of unmarked chinook fry reared in pens and cages, North Pond, Experiment V.

	Stock of chinook salmon			
	Early Run	Late Run		
	Large Live-Car	Pen on Gravel Bottom	Large Live-Car	Small Live-Car
A. Experimental Factors				
Surface area of rearing site (square meters)	0.4	4.0	0.4	0.1
Planting density number of fish per square meter)	65	20	250	250
Length of rearing (days)	49	70	70	70
B. Percent survival	100	49	51	40
C. Production rates (kilograms per hectare per day)				
I. Based on gain in weight of surviving salmon	11	3.4	10	10
II. Based on net gain in weight of salmon	8.5	2.8	4.0	1.4

a highly fertile solution. Consequently, the high oxygen demand of the system can produce low oxygen values in a very short time period when cloud cover is heavy or aeration systems are inoperative. Periods of low oxygen level during mid-October 1972 (Figure 9) caused heavy mortalities during Experiment IV. Other than this instance, and the initial unstable conditions during Experiment IA, aeration units have been able to avoid stratification in the ponds and to maintain oxygen levels sufficient for juvenile salmon.

HEAVY METALS

Data on levels of metals (acid-soluble Cu, Cr, Fe, Na, Ni and Zn) in the pond bottom were available from a study by Gross[28]. Of fifteen locations investigated in the local marine areas, ten were either in or near the North and South fish ponds. Two sampling sites were located in the fish ponds, one each in North and South ponds. The sediments of the oxidation pond, and the fish ponds, contained unnaturally higher amounts of Zinc than all other locations tests (5 samples ranged from 85 to 360 ppm, one sample 25 ppm as opposed to all other areas which ranged from only 2-43 ppm).

There has been no study yet to ascertain if heavy metals in the pond sediments, which undoubtedly represent an area of accumulation due to the operation of the oxidation pond, are entering the food chain of the salmon. A major source of food consumed by the salmon has been two species of amphipod (*Corophium* sp., and *Anisogammarus confervicolus*) but these have not been analyzed for heavy metal content. The higher survival of salmon reared in live-cars and exclusion pens, suggest this possibility.

BIRD PREDATION

Although methods of controlling loss of fish to birds at rearing facilities has been reported extensively in the literature (Blankinship[27]), little data are available on the numbers of fish actually eaten. Thus the pilot project at Humboldt Bay has provided an opportunity to study bird predation on unprotected ponds. These data will allow for comparison of rate of predation on modified ponds, and eventually provide data on the design of production facilities.

Bird predation as estimated by studies undertaken by Blankinship on unmodified ponds showed a variation of from 2-24 percent for coho fingerling (Table 9). The maximum value was associated with an experiment in which fish were under stress, whereas the minimum value was from an experiment in which the density of salmon planted was low and their survival poor. For Experiment VI modifications to North Pond in the summer of 1973 were to deter predation by species of birds that feed along the shore, however, very few birds visited either the modified (North) or unmodified (South) pond, so that effectiveness of the pond modifications could not be assessed from this study.

Table 9. Estimated percentage of salmon planted into North Pond consumed by birds (from Blankinship 1973).

Experiment Number	Species and Size of Salmon	Percentage of Salmon Consumed
IB	Coho fingerling	13[1]
II	Chinook fry	4
III	Coho fingerling	2
	(Stickleback, topsmelt)	(8)
IVA	Coho fingerling	24[2]
IVB	Coho fingerling	12
V	Chinook fry	no study
VI	Coho fingerling	trace

[1] Minimum estimate from limited hours of observation near end of experiment.

[2] Maximum estimate as fish were under stress during parts of the experiment causing higher predator success than occurs on unstressed salmon.

A low predation rate was recorded in experiments with chinook fry. These experiments (II, V) had low survival thus it was not possible to know if bird predation was due to unavailability of chinook to birds or due to differences in behavior of chinook fry as compared with coho fingerlings.

OVER-REARING

There was evidence that we over-reared chinook salmon. During Experiment II, 1/2-meter ring nets were fished at night in North Pond to obtain salmon for study of their food habits. From April 18 through May 24, the average catch per haul was from 2.8 to 5.5 salmon (4-6 hauls per sampling). On May 31, 7 hauls only produced a mean catch of 1.0 salmon. During Experiment IV, studies on fishing ability of 2-cubic feet wire mesh traps with a single funnel entrance caught 28 salmon when fished continuously for 2 days in early April. From 17 May until 25 May, the same traps caught no salmon during this period (8 days).

The exact reason for the sudden loss of salmon in the ponds during late May periods is not known but may be correlated with physical and chemical parameters of the water in addition physiological changes associated with the migratory habits of the species (Reimers[26]). Hydrogen ion concentrations (pH) tended to fluctuate between 7 and 8 during the beginning of these experiments, then rose to stabilize around 9.5 units. Along with this trend, with the general increase in average daily temperature and increased sunshine water temperatures approached 19°C in both Experiments II and V. Smolts recovered in experiments II and V were much larger than the average size reported for smolts of chinook salmon in the Mad River (70 mm fk. len., Taniguchi[29]). Chinook salmon smolting may require a different or more abundant food source than the ponds were providing.

DISEASE

Infectious diseases appeared to be a minor factor as a cause of mortality. This opinion was based on the fact of relatively high survival of salmon reared in floating cages and exclusion pens during experiments when the same stock of salmon reared in the ponds showed low survival. Richness of the diet produced in the ponds for salmon may be a possible cause of mortality (nutritional disease).

FOOD

Gammarid amphipods have been the major food item in the diet of our pond-reared salmon (Powers[30]). Tube-dwelling amphipods (Corphium) undoubtedly will become a major component in the pond fauna when some form of tidal or flow-through operation occurs. Although productivity of the ponds in terms of salmon biomass appears to be declining (Table 4), results from Experiment VI should be discounted. Lack of amphipods during Experiment VI resulted from complete drying of all areas of the pond during a long summer of repair and construction, the elimination of holding areas for amphipods, by replacing the wood construction of fish collecting basin with concrete construction, and finally from a virtual absence of amphipods in Humboldt Bay in September when the ponds were filled for Experiment VI. Undoubtedly the successful management of our wastewater aquaculture system will require as much understanding of the biology of the amphipods as that of the salmon.

CONCLUSIONS

Coho fingerling and chinook fry can be successfully reared in advanced secondarily treated domestic wastewaters mixed with saltwater. The best results in our studies were in mixtures producing 10-15 o/oo salinity (roughly two parts effluent to one part saltwater).

Experiments with coho fingerling reared in late-fall to winter have not carried these salmon to complete smolting of all fish because of our need to have the ponds ready for chinook salmon experiments, our primary target species. Results of these rearing experiments suggest the management possibility of enhancing estuarine survival through such an intermediate period of brackish-water rearing. With completion of a recirculating aquarium system next to our ponds for fish holding and marking, we will begin to test this hypothesis by marking all coho salmon reared, and by holding them for release under the most favorable conditions possible.

The single experiment with coho fingerling in the summer indicated that our fish had not grown to sufficiently large size prior to planting to harvest the marine fishes which had grown rapidly from eggs and larvae introduced from Humboldt Bay. Hayes[25] noted that steelhead in Big Lagoon were mainly yearling fish that had moved into the lagoon during spring months. Future summer rearing will use larger fish (yearling rainbow, steelhead, or coastal cutthroat trout).

Chinook fry have grown rapidly and smolted, although the overall survival achieved would not make the system as a feasible alternative to existing culture methods. The higher growth and survival of chinook reared in pens and live-cars than fish reared in the open ponds, indicate that the full productive capacity of the system has not yet been attained. Recent laboratory studies on bluegill sunfish have indicated the feasibility of increasing the ratio of surface area to water volume for development of more fish food organisms (Pardue[31]). The effect has been noted during studies of artificial reefs in marine waters of Humboldt Bay (DeGeorges[32], Prince[33], Lambert[34]). Any such underwater structures may also have utility in decreasing interspecific competition that we can expect in the ponds (Payne[35], Reimers[26]).

The saltwater-wastewater mixture may have some therapeutic effect as gross observations of our mortalities have not indicated any infectious disease. *Vibrio* can be especially serious in west-coast mariculture (Fryer[20]).

Reliable aeration systems are essential in wastewater mariculture because of the rapidity of oxygen depletion which can occur under certain environmental conditions (heavy fog or cloud cover in particular). Forced-air aeration appears most favorable, and probably will improve pond conditions by stripping ammonia produced by salmon metabolism.

FUTURE PLANS

With recent passage in California of initiative legislation setting up stricter controls on coastline development, the area available for mariculture could be sharply curtailed, especially as we are lacking comparative data on economic returns with other possible types of land uses. Future expansion of mariculture within the confines of the oxidation pond, or into the remaining portion of a now abandoned land-fill refuse site immediately to the west of the Arcata oxidation pond, could take place with land-use conflicts. A master plan for the oxidation pond area has been submitted to the City of Arcata which includes expanded mariculture facilities (Figure 1), developing a similar plan for the land-fill refuse site.

Wastewater stabilization lagoons, fertilized fish ponds, and eutrophic natural waters all contain high numbers of algal cells. Where lagoons and ponds are discharged into receiving waters, water quality standards on BOD and particulate matter may not be attainable without removal of algal cells. We are undertaking studies on mussels as a possible method of biological scrubbing to remove algal cells and pathogens. Successful bivalve scrubbing could reduce the high cost of chlorination required to meet the California State Water Resources Control Board[22] standards for a receiving water where shellfish harvest for human consumption is likely (median total coliform concentration shall not exceed 70 per 100 ml, and not more than 10 percent of the samples shall exceed 230 per 100 ml). Recently, a standard of 0.2 ppm residual chlorine has also been applied, thus increasing the costs of treatment by standard physical-chemical means considerably.

We have become interested in the possibility that reducing the amount of treated wastewaters into Humboldt Bay might actually reduce the biological productivity of this estuarine system. Estuaries in northern California are subjected to hugh freshwater inflows during the winter periods of heavy rainfall. They are, therefore, ecologically adjusted to intermittent periods of reduced salinity. Humboldt Bay has an extremely high flushing volume (Skeesick[36]). Near-shore water of the Pacific Ocean are generally high in nutrients, especially during periods of off-shore upwelling (Allen[37]). Thus the addition of well-treated wastewaters into the system would have beneficial effects. Fortunately, the possibility of reducing a beneficial effect of a well-treated wastewater on a receiving body of water has been recognized by the California State Water Quality Control Board[38]).

In summary, our future program in wastewater maricultre falls into four broad categories. First, we will continue empirical studies to obtain the maximum production of salmon smolts to allow a feasibility study of a production system. Second, we plan to obtain permission for expansion of mariculture ponds into areas adjacent to our present location. Third, we have formed an interdisciplinary team of specialists on the university staff to undertake investigations within their area of competence as an effort in applied ecosystem analysis. Finally, we will expand our preliminary studies on biological methods for removing algae and particulate matter in our effluents through polyculture.

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AQUACULTURE AS A MEANS TO ACHIEVE EFFLUENT STANDARDS

by

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H.G. Chichester and R. LeRoy Carpenter*

INTRODUCTION

Effluent Standards

The Federal Water Pollution Control Act Amendments of 1972** required that all publicly owned treatment works achieve effluent limitation based on secondary treatment as defined by the Administrator of the Environmental Protection Agency. The Act also required that the State classify all stream segments as either effluent limited or water quality limited. Discharges to effluent limited segments must meet the defined[1] secondary treatment requirements whereas discharges to water quality limited segments must generally meet more stringent treatment requirements. Subsequent to publication of the effluent limitations associated with the definition of secondary treatment, the Environmental Protection Agency determined[2] that waste stabilization ponds (lagoons) as presently designed would meet the BOD5 limitation of 30 mg/l, but would not meet the suspended solids limitation of 30 mg/l or the fecal coliform limit of 200/100 ml without improved pond design and operation and additional treatment steps for algae removal and disinfection. Additionally, specific stream segments in many states were designated[3] as water quality limited and require that effluent discharges to those streams have BOD5 concentrations of significantly less than 30 mg/l[4]. As several thousand of these inexpensive and simply operated waste treatment facilities exist, particularly in smaller communities in the less populated areas of the country, it was highly desirable that a means be developed whereby these facilities would meet the various upgraded requirements.

Lagoon Treatment

Lagoon treatment systems rely on biological systems which degrade and stabilize organic compounds in wastewater. A conventional

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**Public Law 92-500

scheme of this process is demonstrated in Figure 1. Prior to lagoon treatment, sewage may receive no treatment at all, mechanical treatment ranging from primary to a high degree of secondary treatment, or extensive biological treatment. Numerous modifications of lagoon design exist and a wide range are in current use.[5,6]

The gross biological steps in this type of common treatment system are: (1) bacterial (anaerobic and aerobic) decomposition of organic wastes resulting in component molecules (plant nutrients) being released into the water; and (2) algal utilization of nutrients producing rapid growth and large populations.[7,8,9,10] The algal phase of the process constitutes the basic mechanism of separation of the nutrients (primarily nitrogen, phosphorous, and carbon) from the water. Proposed mechanisms (Figure 2) to upgrade effluent quality from lagoons tend to be physical in scope and include settling, floculation, and filtration aimed toward the removal of the algal cells. Removal of the algae results in lower concentrations of suspended solids and removal of the elemental constituents comprising their organic mass.[6]

Proper design and maintenance of such a system should provide a high degree of waste treatment. However, these types of treatment mechanisms have some undesirable attributes which are of concern. The algal removal processes involve greatly increased construction, operational, and maintenance costs. In addition, waste products are produced requiring further disposal.[11,12]

Personnel of the Oklahoma State Department of Health have been conducting a preliminary research project since 1970 in an attempt to attack and ameliorate the problems of algal removal and to upgrade the quality of lagoon effluents. The basic assumption of the project is that a biological means of removal exists for the biological phenomenon of algal production. Current systems are biological to the algal removal point. The physical-chemical treatment phase for algal removal is replaced in the system undergoing study by employing a further biological segment based on herbivorus or filter-feeding fishes.

This biological removal concept is presented in Figure 3. Although either an advanced biological treatment system or an advanced physical-chemical treatment system will produce an effluent of high quality, a significant point of this alternative is that the removal of algae through the ecological food chain will produce a useful product in the form of fish as opposed to a product requiring further disposal. Many alternatives for the use of fish are available ranging from reduction to animal food, to sale of live fish for bait, restocking or forage.[13]

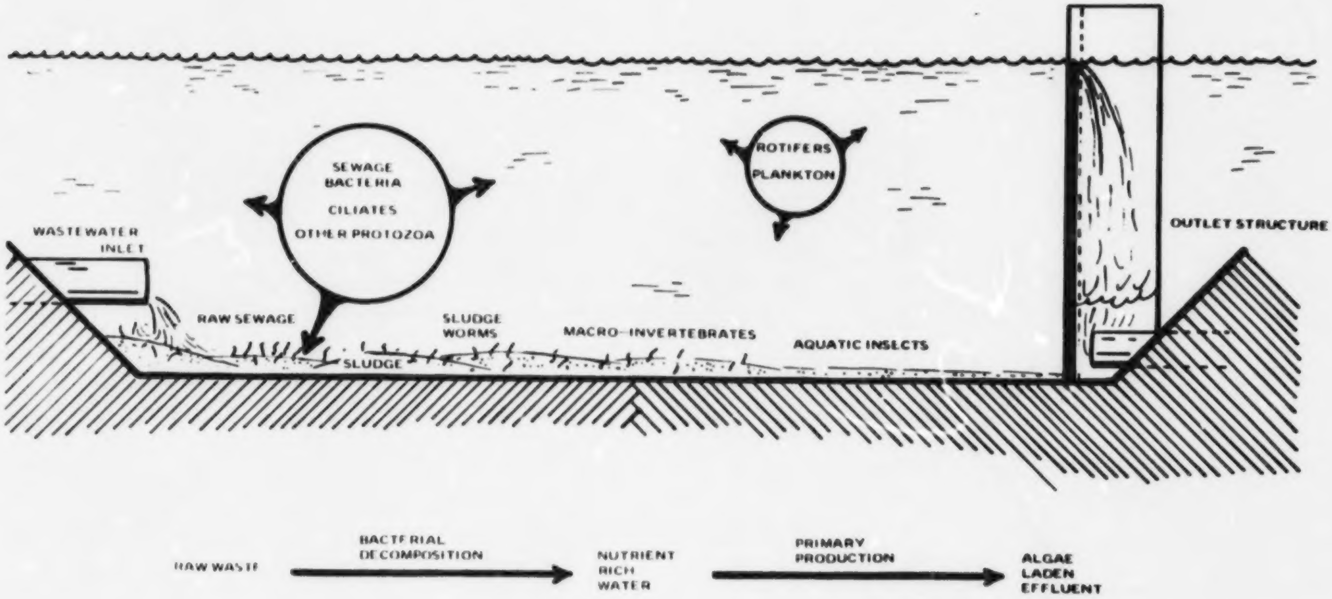

SEWAGE
BACTERIA
CILIATES
OTHER PROTOZOA
ROTIFERS
PLANKTON
WASTEWATER INLET
OUTLET STRUCTURE
RAW SEWAGE
SLUDGE
SLUDGE WORMS
MACRO-INVERTEBRATES
AQUATIC INSECTS
RAW WASTE
BACTERIAL DECOMPOSITION
NUTRIENT RICH WATER
PRIMARY PRODUCTION
ALGAE LADEN EFFLUENT

Figure 1 Schematic Representation of a Typical Waste Oxidation Pond

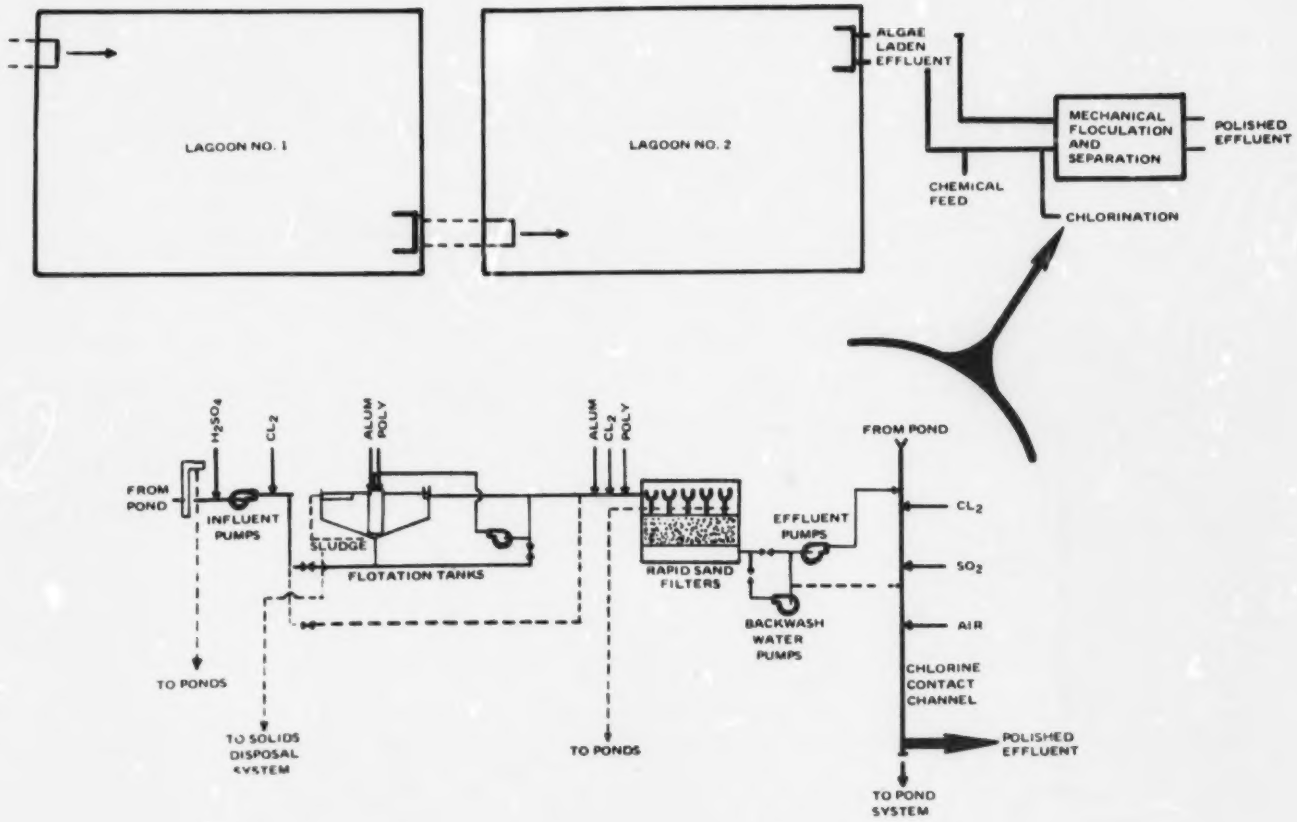
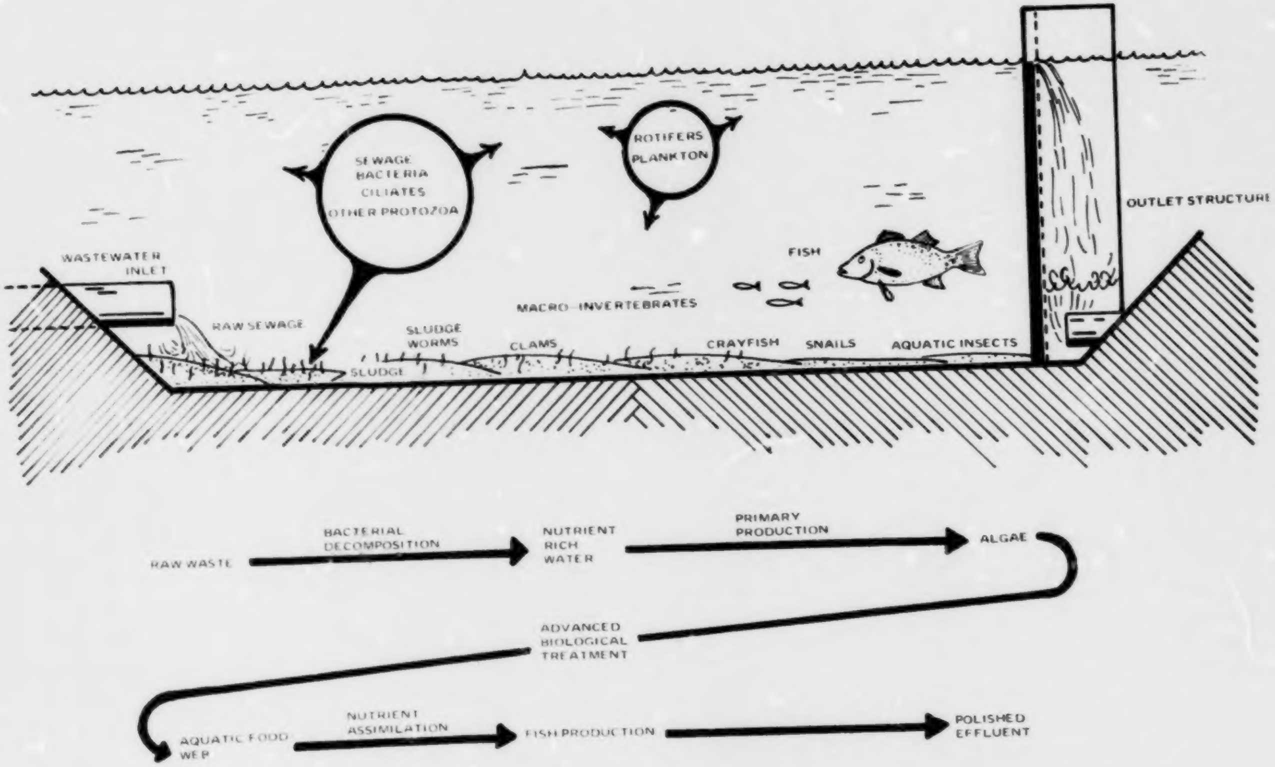

LAGOON NO. 1
LAGOON NO. 2
ALGAE LADEN EFFLUENT
MECHANICAL FLOCULATION AND SEPARATION
POLISHED EFFLUENT
CHEMICAL FEED
CHLORINATION
FROM POND
H_2SO_4
CL_2
INFLUENT PUMPS
ALUM
POLY
SLUDGE
FLOTATION TANKS
ALUM
CL_2
POLY
RAPID SAND FILTERS
EFFLUENT PUMPS
BACKWASH WATER PUMPS
FROM POND
CL_2
SO_2
AIR
CHLORINE CONTACT CHANNEL
POLISHED EFFLUENT
TO POND SYSTEM
TO PONDS
TO SOLIDS DISPOSAL SYSTEM
TO PONDS

Figure 2 Schmatic Representation of a Typical Physical-Chemical Advanced Waste Treatment System


SEWAGE
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PLANKTON
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WASTEWATER INLET
FISH
MACRO INVERTEBRATES
RAW SEWAGE
SLUDGE WORMS
CLAMS
CRAYFISH
SNAILS
AQUATIC INSECTS
SLUDGE
RAW WASTE
BACTERIAL DECOMPOSITION
NUTRIENT RICH WATER
PRIMARY PRODUCTION
ALGAE
ADVANCED BIOLOGICAL TREATMENT
AQUATIC FOOD WEB
NUTRIENT ASSIMILATION
FISH PRODUCTION
POLISHED EFFLUENT

Figure 3 Schematic Representation of Operation of an Advanced Biological Treatment System.

Project History

As the project began to take form in 1970, several species of fish were cultured in the laboratory to determine viability. Thirty gallon aquaria containing wastewater at various levels of treatment were utilized in this preliminary effort. Water was changed on a weekly basis. Species cultured successfully included carp, goldfish, fathead minnow, golden shiner, black bullhead, channel catfish, mosquitofish, bluegill, green sunfish, largemouth bass and _Tilapia nilotica_.

In the spring of 1971 it was decided to apply a larger scale test of fish viability in actual oxidation ponds. Arrangements were made with the City of Oklahoma City to use the Quail Creek Sewage Lagoon system located northwest of Oklahoma City. A diagram of this system is presented in Figure 4. It consisted at that time of a two-cell, serially operated system receiving approximately 750,000 gallons/day of raw domestic sewage. The facility utilized the Hinde Air-Aqua treatment process whereby diffused air was introduced through subsurface air lines. Fish cages were placed near the effluent end of the second cell and anchored to a cable stretched the width of the pond between two air lines. Approximately 200 individuals of promising species, including channel catfish, bluegill, and largemouth bass were placed in the cages where the bench scale viability studies were verified.

SYSTEM DESIGN AND OPERATION

Facility Description

When the facility was originally built, it was planned to eventually utilize three separate sets of the two cell Hinde system. Sufficient funds existed to construct all needed cells in the initial construction. The plan was to add on first facultative lagoon capacity and add aeration as the collection system expanded. By early 1973, about 1 mgd of raw domestic waste was receiving conventional aerated treatment in the first two cell set of the three set system. For experimental purposes, it was determined to utilize the four non-aerated and unused ponds in conjunction with the existing aerated system to form a six cell, serially operated system. (Figure 4) Accordingly, in May, 1973, 25,000 two to four inch channel catfish were stocked in the third cell and an additional 25,000 into the fourth cell. The fifth and sixth cells received 85 pounds (about 1500) golden shiner adults equally divided between the two cells. An additional stocking of approximately 5 pounds of fathead minnows were introduced into the third cell in July, 1973. Also in July, 175 three inch _Tilapia_ _nilotica_ were placed in the third cell.

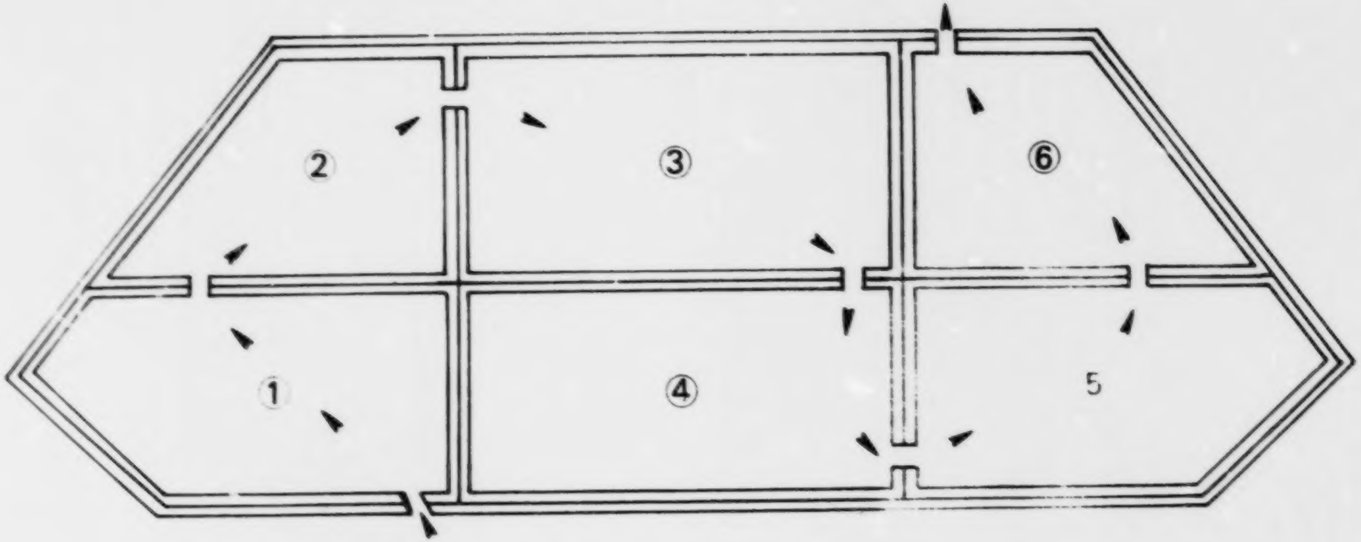

Figure 4 Schematic Representation of Quail Creek Lagoon System

Problems arose in the system when black bullhead, green sunfish and mosquitofish already present in the aerated cells, contaminated the remainder of the system. However, the resulting polycultural situation lent itself to gleaning data on a wider food web than originally intended.

Analytical Methods

Water sampling consisted of removing 2 liters of the raw waste and effluent of each cell on a weekly basis. Standard laboratory analyses[14] were conducted by the Oklahoma State Department of Health and City of Oklahoma City. Bacteriological sampling consisted of removing 100 ml of water from the raw waste and effluent of each cell on alternate weeks. Fish population analyses consisted of a preliminary food habits investigation and biomass estimates. Estimates of biomass were made from seine hauls encompassing a closed known area of each cell. Mean weights were taken for samples of 100 fish and applied to estimated numbers. Food habit data were taken by excising stomachs of various species at the isthmus and duodenal sphincter. Identification of stomach contents was made in the laboratory utilizing standard methodology.

RESULTS AND DISCUSSION

Analysis of Water

A summary of water analyses are presented in Table 1 and are the average of weekly samples from June 6, 19-3 to October 3, 1973. The secondary treatment standard for BOD5 of 30 mg/l was met in the effluent from the second cell and fell significantly throughout the remainder of the system. The remaining secondary standards for suspended solids of 30 mg/l and fecal coliform of 200/100 ml were met in the effluent of the fifth cell.

With respect to suspended solids, it is important to note that from the second cell through the remainder of the system approximately half of the suspended solids were non-volatile. As the newly filled lagoons have sustained considerable bank erosion due to wind action, it is felt that this, when stabilized, will reduce suspended solids. Turbidity data substantiate this contention. Since the volatile portion of the suspended solids is primarily composed of algal cells, a primary target of this research, it is anticipated that the volatile fraction will decrease more rapidly as the system is optimized.

The nitrogen forms are of special interest due to the overall removal efficiency by this system. Ammonia was reduced below 1.0 mg/l N in effluent from the first cell. It is also significant that of

Table 1 Mean Values of Weekly Analyses of Water Samples from the Quail Creek Lagoon System. Samples were Collected June 6, 1973 through October 3, 1973.

Parameters	Raw	1	2	3	4	5	6
Biochemical Oxygen Demand (5 day)	184	47	24	17	14	9	6
Suspended Solids	197	79	71	52	54	26	12
Volatile Suspended Solids	131	54	45	34	27	13	6
Total Nitrogen (as N)	18.94	10.50	7.04	6.65	3.97	3.13	2.74
Nitrite Nitrogen (as N)	0.07	4.54	0.96	0.86	0.34	0.34	0.16
Nitrate Nitrogen (as N)	0.20	1.00	2.31	1.79	0.79	0.31	0.29
Ammonia Nitrogen (as N)	12.67	0.91	0.40	0.31	0.28	0.10	0.12
Organic Nitrogen (as N)	6.10	4.05	3.37	2.69	2.56	2.28	2.17
Total Phosphorous (as P)	9.01	9.87	7.97	5.80	3.66	3.01	2.11
Fecal Coliform/100 ml	3.05×10^6	10880	1380	322	15	15	20
Turbidity	55	15	23	25	42	17	9
pH (standard units)	7.3	7.8	8.2	8.6	8.9	8.4	8.3

*Data reported in mg/1 except where noted.

total nitrogen remaining in the system, the majority from the third through sixth cell was organic nitrogen. This organic fraction was likely present in algal cells. Thus with further refinement of the system, this should ultimately be reduced to even lower levels.

Phosphorus showed good reduction throughout the system to a low of 2.39 mg/l in the final effluent. There were doubtless a variety of factors involved in this reduction, including removal of algal cells. It is likely that at least a part of this removal was due to the formation of a calcium-magnesium-phosphate sludge under the conditions of the high pH values in the third through sixth cell.

The overview of these data indicate a high grade effluent. These preliminary efforts are not seen as maximum efficiency, however further research efforts should attain greater removals.

Food Habits

The preliminary data on food habits allowed general classification of the fishes according to position in the food web. A diagramatic representation is presented in Figure 5.

Tilapia nilotica, fathead minnows and golden shiners were found to consume phytoplankton directly and microcrustacea and insect larvae to a lesser extent. Channel catfish were found to utilize microcrustacea and insect larvae as their principal foods.

This analysis was intended to provide preliminary information regarding the food niche of each species. Future studies should substantiate these preliminary conclusions on food habits.

Biomass

Since no supplemental feeding of fishes occured during the experiment, it follows that fish biomass must have been elaborated from organic constituents present in the system. It is then reasonable to assume that all biomass gained during the observation period represents entrapment of elemental constituents that would possibly have been discharged to receiving waters if the fish had not been present. Thus, the increase in fish biomass was viewed with special interest.

Tilapia nilotica biomass increased from the initial 4 pounds, consisting of 175 individuals (averaging three inches stocked in July 1973) to 163 pounds in October 1973. The harvest consisted of 2339 fish with some individuals attaining lengths of ten inches. It was felt that their planktivorous food habits, the presence of abundant algae, and rapid reproduction rate were the major reasons for this increase. The total biomass of Tilapia was not determined due to procedural difficulties coupled with a sudden temperature drop

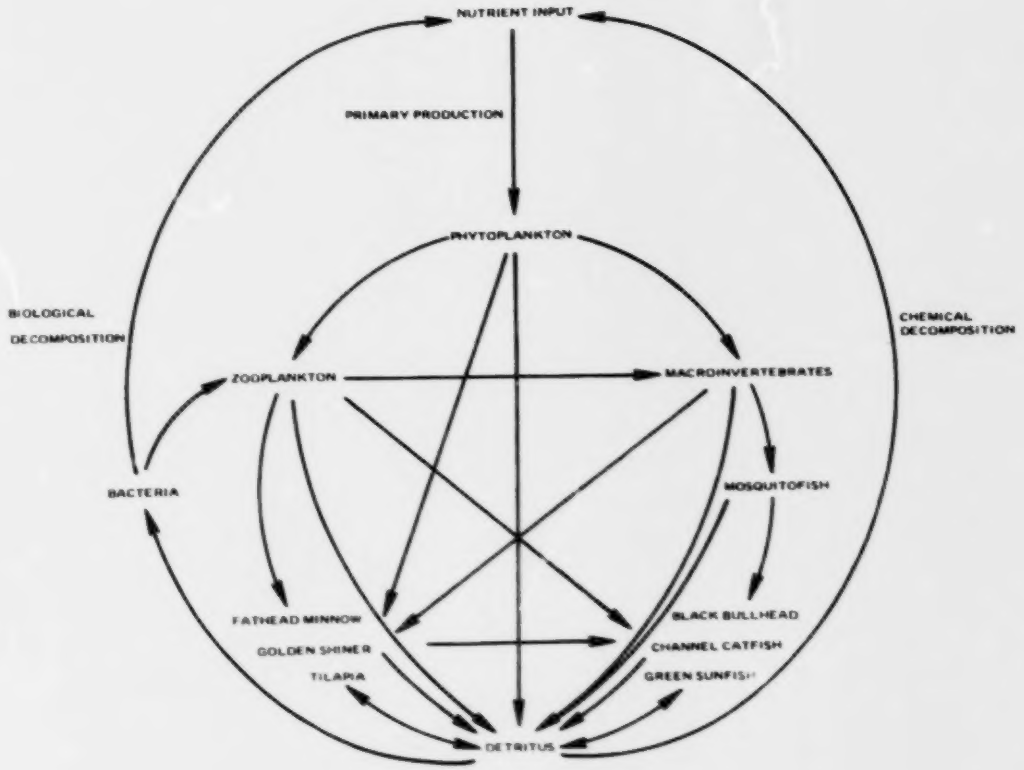
NUTRIENT INPUT
PRIMARY PRODUCTION
PHYTOPLANKTON
BIOLOGICAL DECOMPOSITION
CHEMICAL DECOMPOSITION
ZOOPLANKTON
MACROINVERTEBRATES
BACTERIA
MOSQUITOFISH
FATHEAD MINNOW
GOLDEN SHINER
TILAPIA
BLACK BULLHEAD
CHANNEL CATFISH
GREEN SUNFISH
DETRITUS

Figure 5 General Schematic Food Web of Organisms Inhabitins Cell Three in the Quail Creek Lagoon System, 1973.

which resulted in a large mortality. The biomass reported was determined from Tilapia removed. It is likely that a significantly greater amount of Tilapia were produced and not recovered.

The biomass of channel catfish increased from the initial 600 pounds to an estimated 4400 pounds. However, the channel catfish were observed to gain the majority of this biomass in a six week period from late May 1973 to mid July 1973. Slow growth followed this initial rapid gain. Reasons for this phenomenon are unclear but limited information suggests stunting due to limited food supply. After the virtual stoppage of growth, the system was not operating at maximum efficiency for either water quality improvement or production of fish biomass.

From the initial stocking of 85 pounds of golden shiner minnows, only an estimated 535 pounds were recovered probably due to bullhead catfish predation. It should be noted that only the production of golden shiners in cells five and six was estimated. However, this species was found in moderate numbers in other cells.

Further studies in this area should be oriented toward and include detailed niche identification and utilization to maximize production and resultant upgraded effluent quality by varied species at varied stocking rates.

COST EVALUATION

The replacement of the physical-chemical technology with a biological system producing a marketable product can help defer the cost of treating sewage. Using this concept, cost-benefit analyses were a part of the overall project. Cost figures for the project are presented in Table 2. The cost of additional operation and equipment (line 2) can reasonably be expected to decrease after initial purchases of durable equipment are made. Total estimated cost is 15¢/1000 gallons of domestic sewage.

It is appropriate to note that this comparatively low figure is for an effluent of high quality, surpassing secondary standards usually attainable only through mechanical advanced waste treatment technology. Costs of 40¢ to 45¢/1000 gallons have been reported in mechanical separation.[6] If no monitary recovery at all were made from the fish in the system, this would remain an excellent effluent at a comparatively low price.

The calculations in the lower body of the table project possible cost recovery based on local market value. However, the production estimates are based both on our data and literature sources which suggest the average yield in fertilized systems.[15]

TABLE 2
ESTIMATES OF PROJECTED COSTS & INCOME FROM AN ADVANCED BIOLOGICAL TREATMENT SYSTEM

Costs

180 X 10^6 gallons/six months X .08¢/1000 gallons		=	14,400.00
Additional operational & equipment costs @ rate of $20,000.00/year		=	10,000.00
Fish	50,000 channels at 4¢ apiece	=	2,000.00
	50 gallons minnows at $15.00/gallon	=	750.00
	250 Tilapia at $1.00 each	=	250.00
	$27,400./180 X 10^6 = 15¢/1,000 gallons		$ 27,400.00

Income

50,000 channel catfish at .25¢ each	=	12,500.00
* 1,200 gallons minnows at $15.00/gallon	=	18,000.00
* 18,000 pounds of Tilapia & bullheads at 6¢/pound	=	1,000.00
31,500./180 X 10^6 = 17¢/1,000 gallons		$ 31,500.00

$ 31,500.00
$ 27,400.00

$ 4,100.00/180 X 10^6 = +2.3¢/1,000 gallons

* Estimated production potential.

Using these figures, a return of 2.3¢/1000 gallons is possible. However, even if these figures are high, any sale will be of benefit to the community involved.

As the project proceeds, data will be obtained to more accurately determine the benefits accured from such a biological system operating toward maximum efficiency. The next phase of the project will be to repeat the experiment with no fish present throughout the system.

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PART 4 AGRICULTURE

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AN EXPERIMENT IN THE EUTROPHICATION OF TERRESTRIAL ECOSYSTEMS WITH SEWAGE: EVIDENCE OF NITRIFICATION IN A LATE SUCCESSIONAL FOREST

by

G. M. Woodwell[*], J. Ballard[*], J. Clinton[*],
M. Small[**] and E. V. Pecan[*]

INTRODUCTION

Present practice in treatment of sewage is to release partially treated effluents into water bodies and to rely on natural processes for completion of the treatment. This system of disposal is reaching clear limits as water supplies are exhausted and inland and coastal waters become increasingly polluted. One alternative to this "tertiary" treatment is to use natural or lightly managed terrestrial and aquatic ecosystems singly or in combination as living filters to remove nutrients and release potable water to either ground or surface water channels. The system has the potential for enabling reuse of both water and nutrients.

A series of experiments has been designed at Brookhaven National Laboratory to explore the potential of terrestrial and aquatic systems for recovery of water and nutrients in effluents from primary and secondary sewage treatment plants. Aquatic systems are a pond, a freshwater marsh, a pond-marsh complex and two Phalaris arundinacea meadows[1]. The terrestrial research has been designed around the field-to-forest sere of central Long Island[1-3]. The questions are superficially simple: what is the potential for each of the major communities of the sere for absorption of the solids and nutrient elements in sewage and for the release of "clean" water? How can terrestrial and aquatic systems be manipulated to offer the greatest degree of treatment with the least commitment of fossil fuel energy and the least management?

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[***] Research carried out at Brookhaven National Laboratory under the auspices of the U. S. Atomic Energy Commission with partial financial support from the Town of Brookhaven, Long Island, New York.

THE BROOKHAVEN PROGRAM

DESIGN

The design of the experiments was based on the assumption that the hydrologic cycle for Long Island can be evaluated continuously if precipitation and solar radiation are known[1]. Porous cup lysimeters were used to sample the quality of the percolate that moves below the rooting zone into the ground water; these data will ultimately be used to appraise the water and nutrient budgets of the various plant communities. We are reporting here on the design of the terrestrial segment of the experiment and the early results, some of which have been reported elsewhere[1].

The experiments were established in a section of the Brookhaven National Laboratory site called the Biology Farm (Fig. 1). The terrain is flat throughout the area. Soils are thin, well-to-excessively drained, coarse textured, and podzolic. They have been derived from Wisconsin glacial outwash. Depth to the water table ranges from 5-8 meters. The ground water flows southeastward at a rate of about 13.4 cm/day[1].

Terrestrial plots included an agricultural field, and three communities that together span the field-to-forest sere of central Long Island. The agricultural field was planted in the spring of 1973 with timothy (*Phleum pratense*). The sere was represented by old field, pine forest, and oak-pine forest. The old field community was abandoned after harrowing in the fall of 1972. Most of the field had lain fallow for several years previously. The pine forest was a naturally seeded stand of pitch pine (*Pinus rigida*) that was about 25 years old in 1972. The late successional forest was representative of oak-pine (*Quercus alba*, *Q. coccinea*, *P. rigida*) stands on Long Island[4-7].

Each of these plant communities was divided into three plots, a control and two experimental. The agricultural control plot was fertilized with 1680 kg/ha of commercial 5-10-10 fertilizer. No other control plot received any treatment. The irrigated plots received either primary or secondary sewage at the rate of about 5 cm/week supplementing the normal rainfall.

COMPOSITION OF SEWAGE

The sewage was a mixture of cesspool pumpings (scavenger wastes) obtained from the Town of Brookhaven and sewage from Brookhaven National Laboratory. Two blends were used: one to approximate the effluent from a primary treatment plant; the second, from a secondary treatment plant. A comparison of these blends with other sewage appears in Tables 1 and 2.[1]

Table 1. Comparison of Brookhaven National Laboratory Primary Sewage Blend with Sewage from Primary Treatment Plants in Various Locations.

	BNL "Primary"	Muskegan Mich.	Lancaster Calif.	Allentown Pa.	Port Jeff. N.Y.	Holbrook N.Y.	$\bar{x}$ Domestic	Selden N.Y.
Reference		(8)	(9)	(10)	(11)	(11)	(12)	(13)
MBAS	0.368				1.0			
BOD	372		45	84	73	180	130	240
COD	813		392					
S. Solids	506		90	88	54	75	120	
D. Solids	210		553	482	256	482	560	
Total Solids	716		643	570	310	557	680	228
NH_3-N	10.91	17.21	27	13.5	11.50		15	
NO_2-N	0		0	0.09	0.32			
NO_3-N	0.516	0.89	0.1	0.60	0			
Cl^-	38.1	92.0		92				
Fe	1.97	0.99			1.0			
Mn	0.08	0.15			0.04			
PO_4	4.23	3.04						
SO_4	14.08	22			60			
K^+	8.23	9.63						
Na^+	33.9	65.6						
Ca^{2+}	16.2	85.05	32					
Mg^{2+}	4.13		3					
Cu	0.47	0.93			0	0.15		
Zn	1.33	1.18			2.4			

Table 2. Comparison of Brookhaven National Laboratory Secondary Sewage Blend with Sewage from Secondary Treatment Plants in Various Locations.

	BNL "Secondary"	Muskegan Mich.	Average municipal	Phoenix Ariz.	Bay Park N.Y.	Penn State Pa.	Allentown Pa.	Holbrook N.Y.	Hauppauge N.Y.
Reference		(8)	(14)	(15)	(16)	(17)	(10)	(11)	(11)
MBAS	0.512		6		0.77	0.54			
BOD	131		25	15			35	60	20
COD	280			45	69.9				
S. Solids	168				5-20		54	30	1
D. Solids	172		730				489	490	399
Total Solids	340				384		543	520	400
NH_3-N	5.29	27.4	15.5	24	34	12.8	1.23	25.0	3.0
NO_2-N	0		0.3		0.045		0.47	0.06	0.3
NO_3-N	0.931	0.12	3.5	0.2	0.06	5.1	5.12	0	0.5
Cl^-	33.9	122	130	213	101	4.44	93		
Fe	1.08	0.73			0.22				
Mn	0.03	0.04			0.03	0.10			
PO_4	1.93	3.32	8.3	13	6.2				
SO_4	10.59	29.6	33.3	35.7	165.3				
K^+	5.27	12	15	8		13.8			
Na^+	28.46	80	135	200		52.8			
Ca^{2+}	12.35	129.6	60	82	11.2	32.0			
Mg^{2+}	3.37		25	36		16.3			
Cu	0.24	0.08			0.02			0.15	
Zn	0.70	0.11							

The two blends differed appreciably from one another and from other sewage. Concentrations of phosphorus, sodium, potassium, calcium, magnesium, chlorine, sulfate, and inorganic nitrogen (NH_3, NO_2, NO_3) were slightly lower than in other sewage. BOD and COD loadings were high in both blends used in the Brookhaven work. Sediment content was also high. The ratio of dissolved to suspended solids was very much lower than in municipal effluents. Suspended sediments contained approximately 6% nitrogen and 1% phosphorus. Of the dissolved and suspended sediments, 40 and 80% were combustible and presumably organic.

OPERATION

Two types of spray irrigation equipment were used. In the Primary Agricultural Field sewage was applied by a rotating Aquatower constructed by MacDowell Corporation, DuBois, Pennsylvania. The tower was designed to spray sewage under low pressure close to the surface of the ground. The area covered was 4087 m^2. This equipment has the advantages that the land is not cluttered with piping and is easily accessible to farm machinery, and the low pressure application reduces aerosols. The tower has the disadvantage that it is complex and easily disabled. Conventional aluminum irrigation pipe and fittings were used in all other sprayed plots. Sprinklers were standard Rainbird equipment.

The application rate was about 1 cm (40.4 m^3/ha or 10,691 gal/acre) per day; plots were sprayed Monday through Friday to apply about 5 cm (2 in.) per week. These operations required no more than 75 min/plot/day. Spray operations were conducted throughout the year. In the first winter of operation difficulties encountered during freezing weather prevented operation for several weeks, but improved design will probably allow operation at temperatures well below freezing.

The annual application rates for the chemical and sedimentary constituents of sewage can be approximated from the following relationship:

$$R = K\ S\ C$$

where R = the application rate in kg/ha/yr or lb/acre/yr; K = a constant (5.2 for kg/ha/yr and 4.6 for lb/acre/yr); S = spray rate in cm/week; C = concentration of the substance in question in mg/liter.

Applications rates in the Brookhaven study for N, P and K were 2100, 240, and 215 kg/ha/yr for primary plots and 425, 95, and 140 kg/ha/yr for secondary plots. The total sediment deposit was approximately 3700 and 1750 kg/ha/yr for primary and secondary plots for the spraying regime in use through 1973-74. Spraying was begun in the pine forest and oak-pine forest on July 16, 1973 and in the old field and timothy plots on October 2, 1973.

SAMPLING

Sewage was sampled for chemical composition from the irrigation pipes at the time of application. Amounts applied were measured with buckets distributed in the treated plots[1]. Ground water was sampled for data reported here using 16 wells that tap the upper segments of the ground water[1]. Well pipes were plastic and slotted. A dye study showed that the water moves through the wells with the flow of the ground water.

Percolate was sampled with porous cup lysimeters placed in each plot. A central vacuum system maintained about 1/10 atmosphere tension on each lysimeter continuously. Difficulties with suction equipment caused occasional increases in tension to as much as 6/10 atmosphere.

RESULTS AND DISCUSSION

GROUND WATER

The quality of the ground water varied greatly among the series of ecosystems examined before spray irrigation was started. The most important variation was in nitrate-nitrogen and calcium contents (Fig. 2). Although there was appreciable change in the nutrient concentrations in the ground water through the year of sampling before irrigation, the greatest differences were between the different communities. The mean concentration of nitrate-nitrogen under the forest was about 1/1000 that under the agricultural community; the concentrations of nitrate-nitrogen under the intermediate successional stands were intermediate. The data for calcium followed a similar pattern (Fig. 2). The forest was clearly less of a source of both calcium and nitrogen than the less mature communities. Similar relationships were shown by other major nutrient elements, although in lesser degree.

PERCOLATE

The data on percolation through the first six months of the experiment corroborated the data from the wells. The forest appeared less leaky for most elements than the successional communities. The exception was total nitrogen (Fig. 3), whose concentration in the percolate of the primary plots after five months rose abruptly to the concentration in the spray. A similar pattern was shown in the plots treated with secondary effluent (Fig. 4), but the concentrations reached a maximum after three months and did not match the concentration in the spray. Examination of the relationship between the three forms of nitrogen (Fig. 5) shows that nitrogen in the percolate is in the nitrate form and that the total removed in percolation is approximately the total nitrogen applied. Apparently there is nitrification within the more mature forest during the fall and winter. Whether the nitrification was triggered by the treatment remains to be seen. It did not occur in the pine forest (Figs. 3,4) in either the primary or secondary treated plots. This relationship simply emphasizes the complexity of the nitrogen cycle and the need for greater emphasis on this type of study.

CONCLUSIONS

1. The quality of ground water on Long Island is affected profoundly by the vegetation. The more mature, late successional forest produces percolation into the ground water that is normally low in the major nutrients, especially nitrate-nitrogen. Agricultural communities and plant communities of early succession are leaky by comparison.

2. The conversion of ammonium-ion nitrogen to nitrate appears to occur in the irrigated stands of oak-pine forest during winter but not in the pine stands. The question of whether irrigation has triggered nitrification is unresolved.

3. There is little question that natural and agricultural communities can be used to treat sewage. There is need, however, to examine how harvest of nutrients is to be accomplished most effectively to avoid the accumulation of salts to the point where losses equal inputs. The assumption seems justified that any treatment system will require more than one plant community but there is little basis at present for speculation as to the combinations that will prove most useful.

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FIGURE LEGENDS

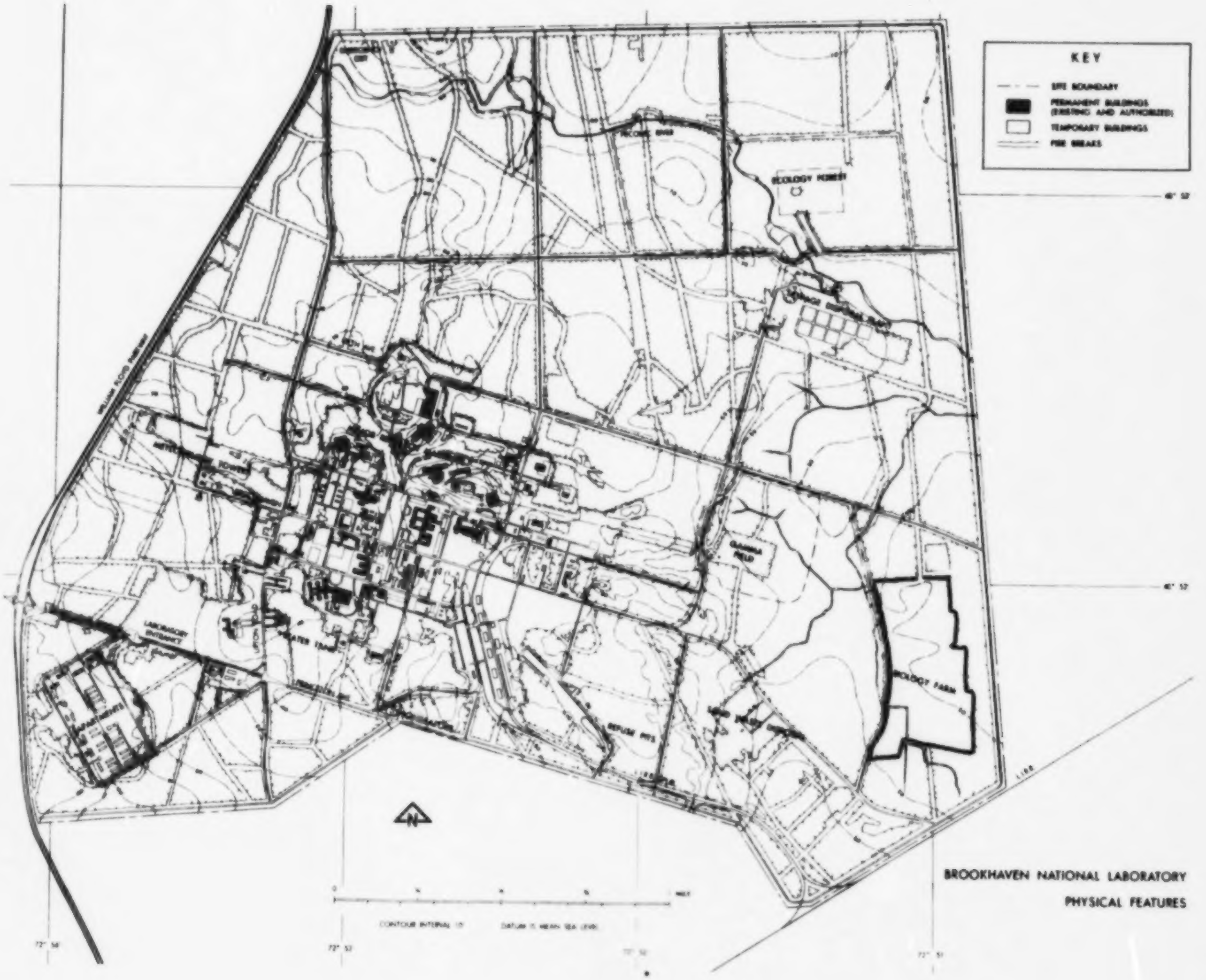
Fig. 1. The Brookhaven National Laboratory site. The experiment is at the Biology Farm in the southeastern corner of the site. Sewage is prepared from scavenger wastes and untreated Laboratory sewage and pumped to holding ponds at the experiment.

Fig. 2. Nitrate-N and Ca content of ground water under major plant communities of the field-to-forest sere, Brookhaven, New York. The ground water flows laterally at about 13 cm/day. Each curve represents a single well.

Fig. 3. Total nitrogen concentrations in primary sewage and in soil percolate under the plant communities of the sere.

Fig. 4. Total nitrogen concentrations in secondary sewage and in soil percolate under the plant communities of the sere.

Fig. 5. Concentrations of ammonium-nitrogen, nitrate-nitrogen and total nitrogen in primary sewage applied to terrestrial communities and in percolate leaving the rooting zone. Key: S = sewage, P = pine forest percolate, F = oak-pine forest percolate, A = agricultural field percolate, O = old field percolate.


KEY
SITE BOUNDARY
PERMANENT BUILDINGS (EXISTING AND AUTHORIZED)
TEMPORARY BUILDINGS
FIRE BREAKS
40° 52'
ECOLOGY FOREST
ECOLOGY FARM
N
0
1 MILE
CONTOUR INTERVAL 10'
DATUM IS MEAN SEA LEVEL
72° 54'
72° 53'
72° 52'
72° 51'

BROOKHAVEN NATIONAL LABORATORY
PHYSICAL FEATURES

FIGURE 1

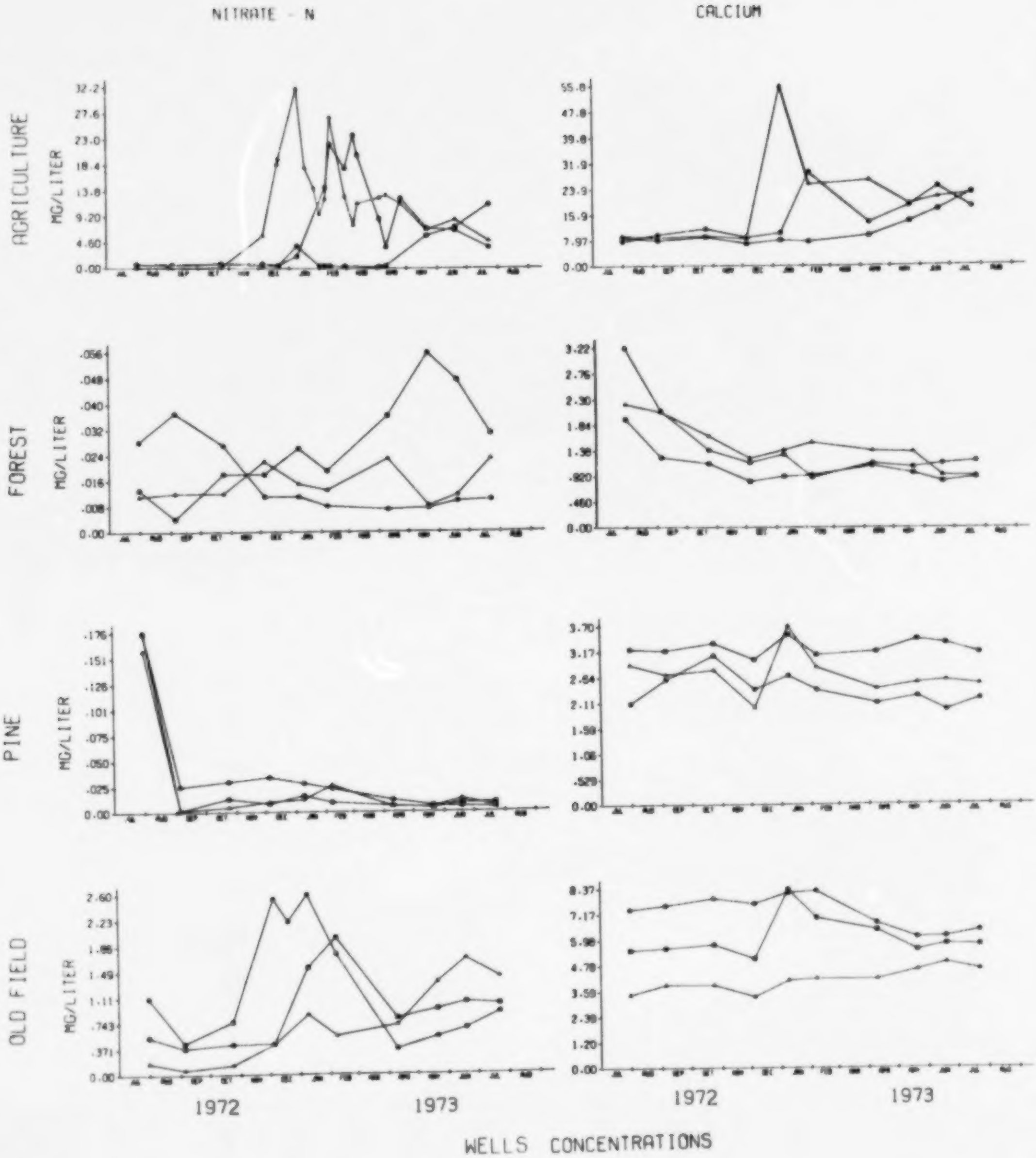
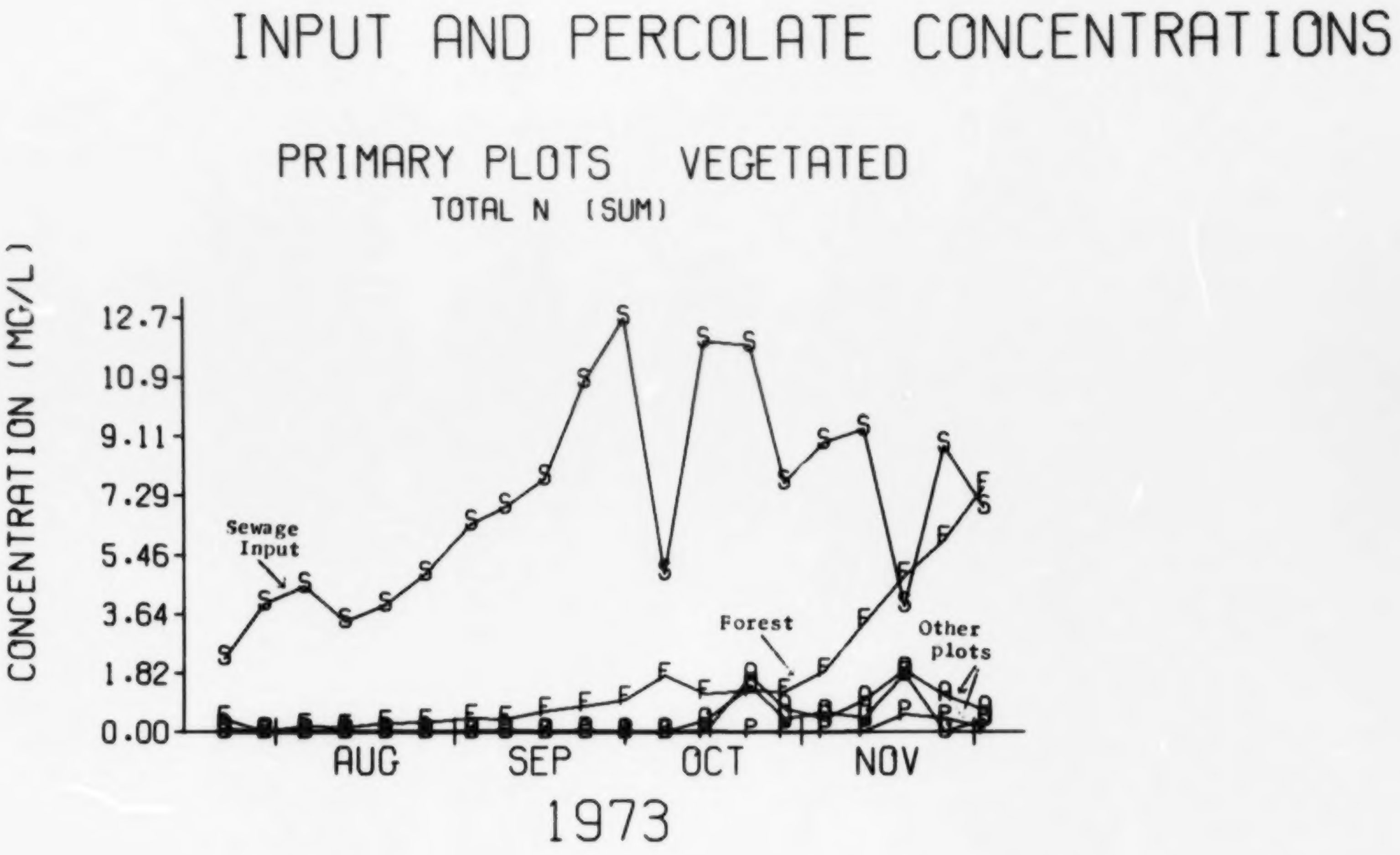

NITRATE - N
CALCIUM
AGRICULTURE
FOREST
PINE
OLD FIELD
MG/LITER
32.2
27.6
23.0
18.4
13.8
9.20
4.60
0.00
55.8
47.8
39.8
31.9
23.9
15.9
7.97
.056
.048
.040
.032
.024
.016
.008
3.22
2.76
2.30
1.84
1.38
.920
.460
.176
.151
.126
.101
.075
.050
.025
3.70
3.17
2.64
2.11
1.58
1.06
.529
2.60
2.23
1.86
1.49
1.11
.743
.371
8.37
7.17
5.98
4.78
3.59
2.39
1.20
1972
1973
WELLS CONCENTRATIONS

FIGURE 2



INPUT AND PERCOLATE CONCENTRATIONS

PRIMARY PLOTS VEGETATED

TOTAL N (SUM)

FIGURE 3

INPUT AND PERCOLATE CONCENTRATIONS

SECONDARY PLOTS VEGETATED

TOTAL N (SUM)

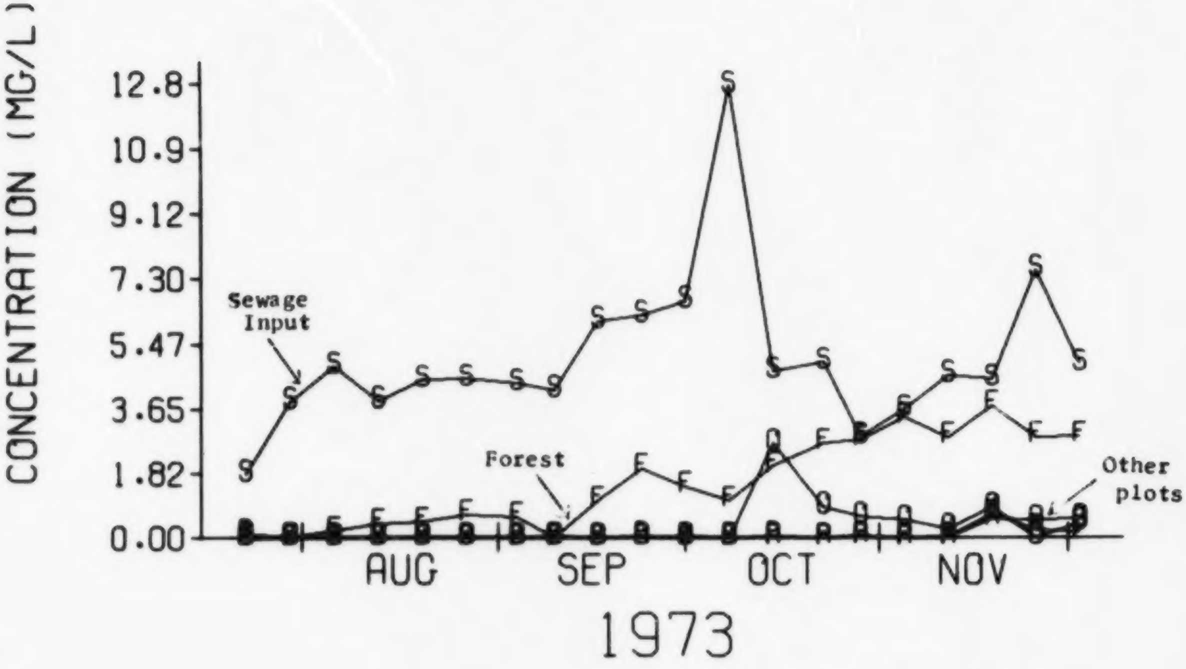
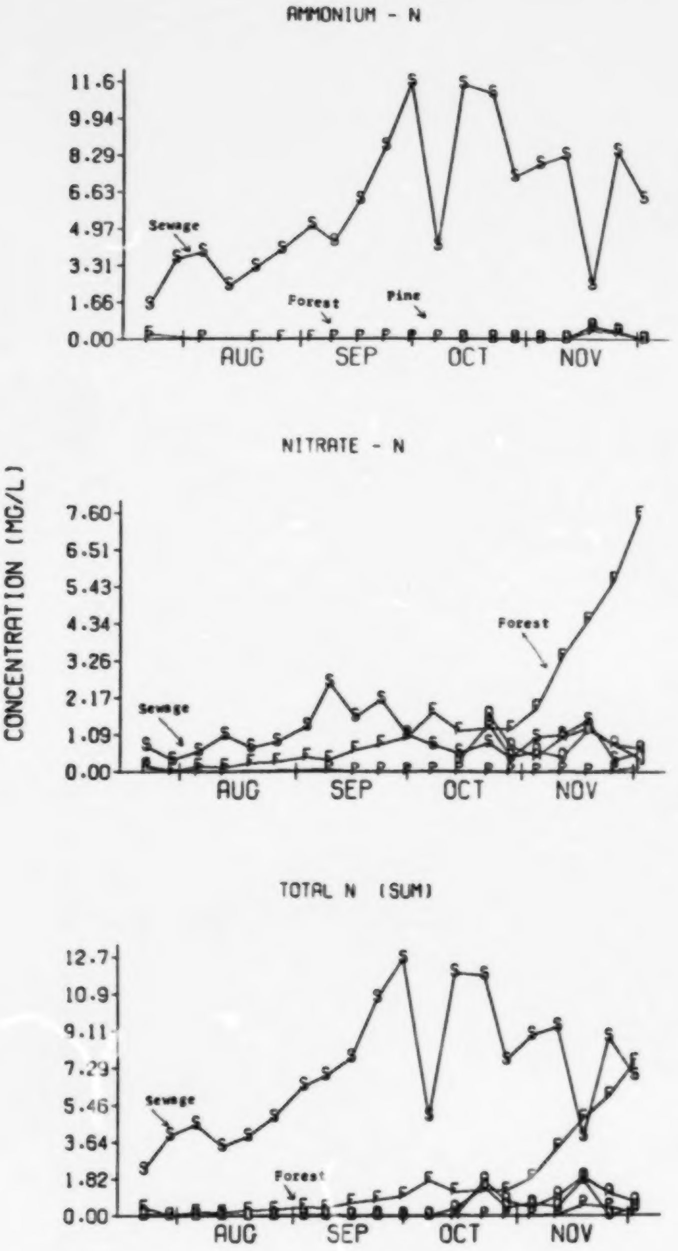


FIGURE 4



1973

INPUT AND PERCOLATE CONCENTRATIONS

PRIMARY PLOTS VEGETATED

FIGURE 5

IRRIGATION WITH WASTEWATER AT BAKERSFIELD, CALIFORNIA

by
Ronald W. Crites*

INTRODUCTION

Wastewater has been used to irrigate cropland at Bakersfield, which is located at the southern end of the San Joaquin Valley in the central part of California, for more than 60 years. The purpose of this paper is to document the principal features of this successful operation by tracing its historical development, current operation, and environmental effects. Crop yields are related to the characteristics of the effluent applied and compared to typical yields in Kern County. Future plans are discussed and the applicability of the data and experience gained from this long-established operation to other similar operations is delineated.

Normal annual precipitation in the Bakersfield area is about 6 inches. Because it occurs mostly from December to February, irrigation is necessary for summer crop production. Since irrigation began in the area, agriculture has been a major industry.

HISTORICAL DEVELOPMENT

Although the city has three wastewater treatment plants, most of the domestic wastewater from the city is treated at Plants No. 1 and No. 2, located southeast of the city. The farmland adjacent to these two plants has received wastewater since 1912, when the first portion of the city's sewerage system was constructed.[1] The outfall sewers ended in an open ditch from which untreated wastewater was taken for irrigation.

In 1933, the California Department of Health conducted a state-wide survey of wastewater irrigation systems.[2] The report for Bakersfield was that 58 acres of cotton, 480 acres of wild grass, and an unspecified acreage of pasture were being irrigated with untreated wastewater.

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*Project Engineer, Metcalf & Eddy, Inc., Palo Alto, California

In 1939, a 9.0 mgd primary treatment plant (Plant No. 1) was constructed. In 1952 the plant was severely damaged by an earthquake and the effective capacity was reduced to 4 mgd.[3] In the same year, a 16 mgd primary plant (Plant No. 2) was constructed approximately 2 miles to the south. The city continued to dispose of the effluent from both plants on adjacent farmland, now owned by the city and leased to a grower. In 1948, a secondary treatment plant was built adjacent to city Plant No. 1 by the Mount Vernon Sanitation District. Although the effluent is also used nearby to irrigate crops (pasture, alfalfa, and barley), the Mount Vernon operation will not be discussed further.

In 1956, Merz[4] made a field visit and reported corn, cotton, alfalfa, sorghum and grass being grown on 2,000 acres. In 1959, Scott[5] reported cotton, field corn, milo maize, sugar beets, barley, and permanent pasture as crops on the 2,500 acre farm. Finally in 1973, the crops grown were cotton, corn, barley, alfalfa, and pasture grass.[6] Although the total farm acreage is 2,500 acres, only about 2,400 acres can be irrigated for crop production.

CURRENT OPERATION

Presently the city of Bakersfield leases the farmland to Mr. Joe Garone for $40,000.00 annually or $16.00 per acre. Mr. Garone takes all the 13 mgd of effluent from the two primary plants throughout the year. He rotates his crops, maintains ditches, and controls the tailwater from irrigation. The major summer crops are field corn and cotton, with only 80 acres being planted to alfalfa. Mr. Garone is in his fourth year as leasee; consequently, he is still releveling certain areas and leaving some plots fallow to reduce weed problems that he inherited.

Wastewater Characteristics

The wastewater is primarily domestic in nature, with only a few poultry-processing plants discharging high-BOD wastes to Plant No. 1. The characteristics of effluents from Plant No. 1 (3.5 mgd) and Plant No. 2 (9.5 mgd) have been combined and a typical blend of constituents that would be found in the irrigation water is given in Table 1.

The domestic water supply for Bakersfield is obtained from deep groundwater wells. Nitrate concentrations up to 18 and 19 mg/l as nitrate are found in some of these wells.

Table 1. Characteristics of Treated Effluent at Bakersfield

Characteristic	Values in mg/l except as noted
Flow, mgd	13.0
BOD	150
Suspended solids	48
Total nitrogen as N	28
Ammonia nitrogen as N	25
Organic nitrogen as N	3
Nitrate nitrogen as N	0
Phosphorus as P	6.2
pH, units	7.2
Total dissolved solids	380
Chloride	60
Sulfate	90
Bicarbonate	220
Calcium	15
Magnesium	18
Sodium	112
Potassium	12
Percent sodium	65
Sodium adsorption ratio	4.4
Boron	0.5

The mineral quality of the combined primary effluent is quite suitable for irrigation. The total nitrogen level of 28 mg/l causes some problems in the growing of cotton, as will be discussed later. The percent of sodium is relatively high at 65 percent; however, it is not critical. The total dissolved solids (TDS) concentration is not a problem for any of the crops grown.

Site Characteristics

The topography is very flat, with the fields graded for surface irrigation. General drainage is from north to south with sump pumps along the southern end to return tailwater to storage ponds (See Figure 1). Soils range from fine sandy loams to clay loam. The soils are

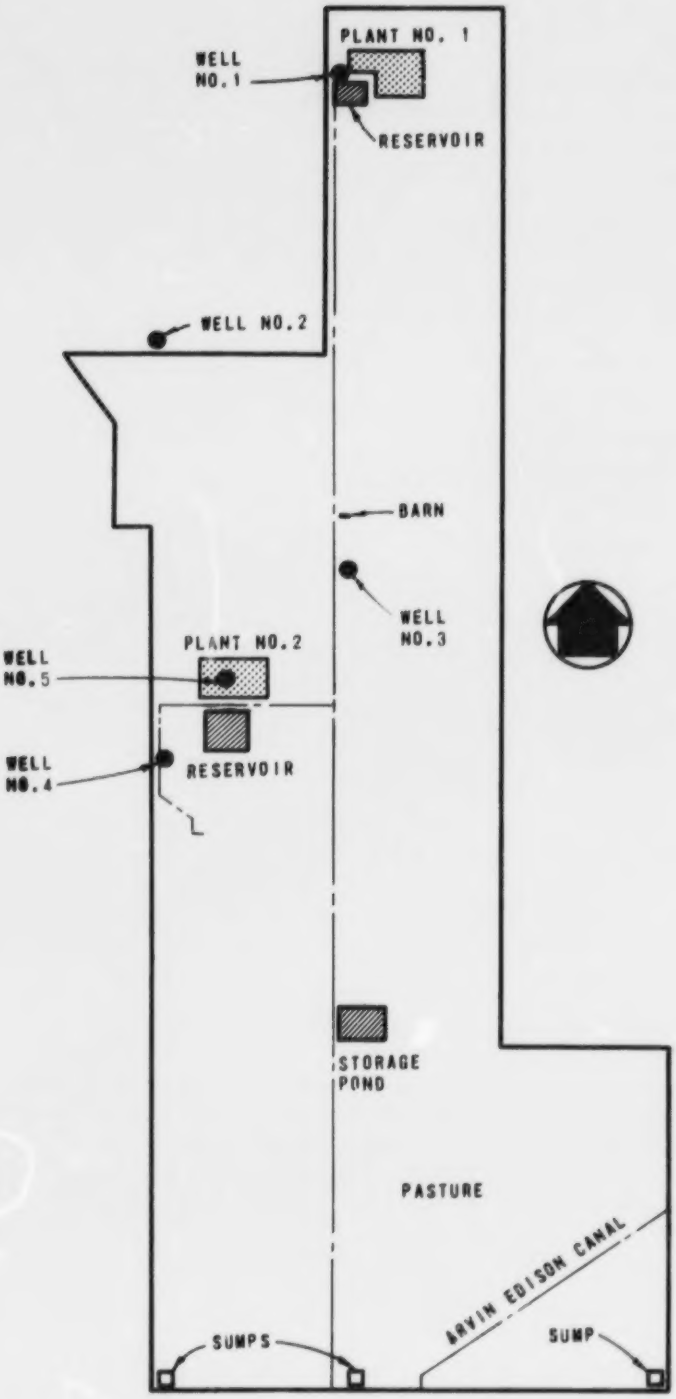
PLANT NO. 1
WELL NO.1
RESERVOIR
WELL NO.2
BARN
WELL NO.3
PLANT NO.2
WELL NO.5
WELL NO.4
RESERVOIR
STORAGE POND
PASTURE
ARVIN EDISON CANAL
SUMPS
SUMP

FIGURE 1 SOUTHEAST BAKERSFIELD CITY FARM

generally alkaline and poorly drained with dense clay lenses at depths ranging from 10 to 15 feet below the surface. This clay barrier produces perched water in areas where it is continuous and reduced percolation in areas where it is not.

Permanent groundwater aquifers exist at approximate depths of 100 to 200 feet and at 300 feet. The two are separated by a clay barrier, and the confined lower aquifer is used for water supply. The deep wells on the farm, as shown in Figure 1, produce water for supplemental irrigation water. The quality, however, is inadequate for potable uses as a result of high TDS and nitrate content.

Crop Irrigation

All crops are irrigated by surface methods. Corn and cotton are irrigated using the ridge and furrow method; and pasture, barley, and alfalfa using the border strip method.

The nitrogen needs of the crops, the usual amounts added by commercial fertilizer in Kern County, and the actual nitrogen loading rates are given in Table 2. As can be seen, the nitrogen applied more than meets the nitrogen uptake of all crops. For both cotton and alfalfa, the nitrogen loading is more than twice that which can be utilized. Because alfalfa is a legume, it is not usually fertilized with nitrogen. For cotton, excess nitrogen fertilizer results in excess vegetative growth at the expense of fruitive growth, and subsequent yields are descreased.

Table 2. Nitrogen Loadings at Bakersfield Compared to Typical Fertilizer and Uptake Rates

Crop	Actual Nitrogen loading rate from effluent, lb/acre/yr	Typical commercial fertilizer rate, lb/acre/yr[a]	Nitrogen uptake, lb/acre/yr
Alfalfa	371	--[b]	100-150
Barley	139	80-90	75
Corn	252	200-250	150
Cotton	277	100	100
Pasture grass	371	100+	150-250

a. Average for Kern County.

b. 50 lb/acre/yr of P_2O_5 added by some growers.

Liquid loading rates and loadings of BOD, potassium, and phosphorus are listed in Table 3. The irrigation and agricultural practices will be discussed individually for each crop.

Table 3. Loading Rates for Primary Effluent at Bakersfield

Crop	Liquid loading rate, ft/yr	BOD loading rate, lb/acre/yr	Potassium loading rate, lb/acre/yr	Phosphorus loading rate, lb/acre/yr
Alfalfa	4.9	1,985	159	82
Barley	1.8	730	58	30
Corn	3.3	1,300	107	55
Cotton	3.7	1,500	120	62
Pasture grass	4.9	1,985	159	82

Alfalfa - Alfalfa is a perennial crop that is irrigated from March to October at about 2-week intervals. Cuttings are made approximately monthly during this period. As noted in Table 2, some growers in Kern County fertilize with 50 pounds of P_2O_5 per acre, which is equivalent to about 11 pounds of elemental phosphorus.

Alfalfa will take up to 21 pounds annually of phosphorus and 110 pounds of potassium.[7] Both uptakes are more than met by the application of effluent.

Barley - Planted in mid-December, barley can be harvested for grain in mid-May or early June, or it can be grazed. Mr. Garone allows his cattle to graze about 160 acres of barley in rotation with other pastureland and the feed lot. Barley is pre-irrigated in November, and then irrigated once every 2 weeks from mid-March to harvesting. Barley that is grazed is generally disced under by June 1 to allow the field to be prepared for planting of corn.

Corn - Planted in early June or late May, corn requires a great deal of water during its 90 to 100 days of growth. Corn is generally irrigated at a rate of nearly 3 in./wk. Mr. Garone harvests his field corn in September for ensilage and hauls it to his feed lot.

Cotton - Cotton is an annual crop, planted in mid-March and harvested in mid-October. Land to be planted is pre-irrigated with about 15 inches of effluent in February. Irrigation of the cotton begins in June and is ceased by September 1. Mr. Garone has problems growing cotton because irrigation with 3.7 feet of effluent adds nearly 3 times as much nitrogen as needed. This results in excessive plant growth and reduced yields. To compensate, careful management is required, including steps to (1) thin the cotton more than usual, (2) stress the plants between irrigation periods to stimulate fruitive growth, and (3) blend effluent with well water to reduce the total nitrogen concentration. Despite the use of these techniques, cotton yields are generally 20 percent less than the county-wide average.

Pasture - Mr. Garone raises pasture grass on the remaining portions of the farm. These 750 to 800 acres represent the poorest soils and also receive the tailwater from other areas. Pumps and small sumps exist at the south side of the pastureland to return tailwater to a storage pond. The pastureland supports between 1.5 and 2 animal units per acre. The land is irrigated every 10 to 14 days throughout the year. In the past, excessive irrigation has led to ponding in the southern portion of the pastureland, with problems from odors and mosquito propagation resulting.

Crop Yields

Yields resulting from irrigation with primary effluent, typical prices commanded by each crop in 1973, and the economic return per acre are presented in Table 4. The double-cropping practice of barley followed by field corn will yield the highest gross economic return but will also require the most field preparation, cultivation, and management.

Table 4. Crop Yields and Economic Return

Crop	Yield, lb/acre	Typical price, \$ per pound[a]	Economic return, \$ per acre
Alfalfa	16,000	0.025	400
Barley	3,000-5,000	0.045	135-225
Corn	36,000-60,000	0.0075	270-450
Cotton	600-800	0.35	210-280

a. Based on 1973 prices.

Alfalfa yield is equal to, if not slightly higher than, the county-wide average of 6 to 8 tons per acre. Barley yield is also approximately the same as the county-wide average. Corn yields on a county-wide basis are 20 tons per acre. Mr. Garone[8] finds that up to 30 tons per acre are possible on the heavier loam soils, while 18 tons per acre is a typical yield on the sandy alkali loam soil.

Yields for cotton are typically 20 percent less than county-wide averages as a result of the excess nitrogen and the compensatory measures required, and because of the relatively poor quality alkali soil. To increase yields, Mr. Garone is applying gypsum to areas that are strongly alkaline. When Merz visited in 1956, he reported that gypsum was applied at 4 tons per acre.[4] He also reported a cotton yield of 1.82 bales per acre for 1955 compared to the current yield of approximately 1.5 bales per acre.

Economic Considerations

Although the original cost of the land was probably less than $100.00 per acre because of the poor quality of the soil, it is now worth close to $1,000.00 per acre. Mr. Garone's lease from the city amounts to about 20 percent of the operation and maintenance costs for the two treatment plants, which are approximately 5 cents per thousand gallons.[7] Thus the city's property has appreciated in value, while 20 percent of their annual budget for two of their plants is repayed from the farming operation.

The cost of irrigation water from the local canal is about $5.00 per acre-foot at the canal side, and the cost of pumping groundwater is typically $15.00 to $20.00 per acre-foot. If the $40,000 lease applied only to the 14,500 acre-feet of effluent supplied, the cost of effluent would be $2.76 per acre-foot. This economical price must be balanced against the requirement of managing excess flows in the winter.

HEALTH EFFECTS

Merz[4] reported that no diseases have been traced to effluent use, although there have been problems with flies and mosquitoes. The current Superintendent of the city's treatment plants, Mr. James Groves,[9] reiterated that no diseases have been associated with the operation. Mosquitoes are a problem wherever water is allowed to pond and stagnate, so that keeping the excess water moving is a major management problem. The two equalizing reservoirs

(5 and 10 acres in size) and the storage pond for tailwater are periodically sprayed to control mosquito propagation.

Current public health regulations for irrigation of fodder, fiber, and seed crops are that the quality of reclaimed water shall be equivalent to primary effluent.[10] No disinfection of the effluent is required, and none is provided at the two plants. Dairy cows are not allowed to graze pastures irrigated with nondisinfected effluent; however, beef cattle are not restricted.

ENVIRONMENTAL EFFECTS

A complaint by the Regional Water Quality Control Board that the irrigation operation was contaminating the groundwater with nitrates led to an investigation of the groundwater quality by Metcalf & Eddy.[6] Six shallow wells were drilled and sampled in late 1972. Perched water was found at the 11- to 12-foot depth in 3 wells and not at all in the other 3 wells (drilled 30 feet deep). Nitrate concentrations ranged from 4 mg/l as nitrate in the center of the farm to more than 600 mg/l nitrate directly beneath the old site of the sludge drying beds at the Mount Vernon plant.

The Regional Board conducted a sampling program in September 1971 and found nitrate concentrations ranging from 0.4 mg/l to 68 mg/l as nitrate. These wells were along the western edge of the farm, which is in the direction of flow of the unconfined aquifer. These wells were sampled at depths of 80 to 170 feet.

No firm conclusions relating effluent irrigation to groundwater nitrate levels could be drawn from either investigation. The confined aquifer below the farm (300 feet deep) has nitrate concentrations of 50 to 60 mg/l as nitrate.[6] However, many wells, both deep and shallow throughout the Bakersfield area have high nitrate and TDS concentrations. The groundwater hydrology of the area is complex and groundwater quality is influenced not only by irrigation practices, but also by oilfield well injections, natural occurrences of salt, and cattle feedlots. A more detailed investigation of groundwater quality is in the planning stage for 1975.

FUTURE PLANS

Because of the problems with mosquitoes, the ponding of excess irrigation water in the winter, and the poor quality of effluent from Plant No. 1, the state has required that the city investigate alternative treatment and reuse systems. Metcalf & Eddy conducted this investigation and prepared a project report.[3] Treatment and reuse alternatives included (1) overland flow treatment and disinfection with effluent discharge to an irrigation canal, (2) treatment and continued irrigation using more land, (3) treatment and reuse in recreational lakes, (4) treatment and groundwater recharge followed by withdrawal for discharge to irrigation canals, and (5) advanced wastewater treatment with discharge to the irrigation canal. It should be noted that for Alternative 2 the Regional Water Quality Control Board proposed a requirement of secondary treatment prior to irrigation, which is more restrictive than the State Department of Health regulations require. Although all alternatives would involve beneficial reuse, Alternative 1 was the most cost-effective.

Current plans (provided the regulatory agencies approve the concept) are to consolidate Plant No. 1 and No. 2 and construct a pilot overland flow (spray-runoff) system. The design of this first stage could begin as early as July 1974.

SUMMARY

Much can be gained by studying the successful operation of wastewater irrigation at Bakersfield. Storage of effluent, control of tailwater, and circulation of water to avoid shallow ponding are successful management techniques that have been developed. Control of the farming operation by an experienced grower who applies proper amounts of water for crop needs, adds soil amendments as needed, and compensates for excess nitrogen (in the case of cotton) is another lesson.

Crop yields vary from slightly below to well above the county-wide average. These yields are greatly affected by the soil characteristics as well as the effluent characteristics. The alkaline soil is still being reclaimed by additions of gypsum in certain areas.

ACKNOWLEDGEMENTS

The cooperation of Mr. Joe Garone and Plant Superintendent Mr. James Groves is gratefully acknowledged. The typical county-wide yields and prices were provided by Mr. Dave West of the Agricultural Extension Service in Bakersfield.

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NUTRITIVE VALUE OF AEROBICALLY TREATED LIVESTOCK AND MUNICIPAL WASTES *

by

D. L. Day and B. G. Harmon **

Numerous methods of utilizing livestock wastes (manure) have been studied, including spreading it on the land as a fertilizer; using it to produce fuels, make building materials; composting it for a soil ammendment; and various methods of processing for refeeding. Using livestock wastes to produce feedstuffs offers two obvious advantages: minimizing environmental pollutants and realizing a new source of nutrients.

The acceptance of procedures that utilize treated wastes as nutrients requires some explanation and education. However, refeeding animal excrement, either inadvertently or intentionally, has been practiced to some extent since the domestication of animals.

Before vitamins and amino acids were understood, livestock producers knew that the fastest-growing and most-efficient hogs were those that "followed" the cattle. The hogs gleaned the cattle manure for undigested grain, but they also utilized the vitamins and monocellular protein. Also, young pigs needed more iron than they received from sow's milk. Access to the sow's feces containing high levels of biologically available iron prevented anemia in the pigs. Modern livestock rations are nutritionally balanced to avoid such problems; however, processed wastes can supply some of the nutrients and thus help resolve the waste-management problem.

This paper deals with the aerobic treatment of metabolic wastes for utilization in diets of livestock as a source of various nutrients but especially protein as amino acids. Aerobic treatment of sewage is a long-known process by which microbial activity is intensified (Arden and Lockett, 1914). By its nature, this is a low-odor process; but it requires external power for the aeration. In recent years, the aerobic treatment of livestock wastes has gained popularity mainly because of its low-odor characteristic (Jones *et al.*, 1971). The power requirement for continuous aeration is the main disadvantage.

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NUTRIENTS IN MUNICIPAL ACTIVATED SLUDGE

Hurwitz (1957) reported the amino-acid, vitamin, and mineral content of dried activated sludge from the Metropolitan Sanitary District of Greater Chicago, Table 1. This product was successfully used as a feed additive for swine, poultry, and sheep and beef cattle in experiments at the University of Illinois in the 1950's (Schendel and Johnson, 1954; Firth and Johnson, 1955; and Hackler *et al.*, 1957). These studies established the value of vitamin B_{12}, nitrogen, and unidentified growth factors in the activated sludge. There were, however, some severe diarrhea problems with steers and lambs (Hackler, 1958).

Work conducted at Bangalore, India in the 1960's showed that half of the vegetable and animal protein supplements in the diet for chicks can be supplied by activated sludge (Pillai *et al.*, 1967). Table 2 shows an analysis of the sludge. The experiment was carried through the egg-laying period, and significantly more eggs were laid by the birds on the sludge-supplemented diet than by those being fed a typical diet. Pillai *et al.* (1952) also reported that the purification processes inherent in the aerobically treated activated sludge eliminated pathogenic bacteria causing typhoid, cholera, and dysentery.

A method of producing organic molasses by the hydrolysis of activated sewage sludge is reported by Bouthilet and Dean (1970). The molasses had an amino-acid content favorable for animal feeds, Table 3; and it was successfully fed to weanling rats at the 5- and 10-percent level; feeding at the 25-percent level produced detrimental results.

An analysis of grab samples of activated sludge from the Urbana, Illinois Municipal Waste-Treatment Plant is given in Table 4.

NUTRIENTS IN AEROBICALLY TREATED LIVESTOCK WASTES, UNIVERSITY OF ILLINOIS PROJECTS

Since 1963, a research project has been underway at the University of Illinois at Urbana-Champaign on managing livestock wastes by means of aerobic treatment in oxidation ditches beneath slotted floors in livestock buildings, a modified form of the Pasveer oxidation ditch (see Figure 1). The original emphasis was on odor control and low-labor waste management; but in recent years, the microbially enhanced oxidation ditch mixed liquor (ODML) has been studied as a source of nutrients for livestock.

An analysis of a sample of swine ODML in 1967 showed a high potential for a protein supplement, Table 5. The refeeding implications are obvious. Methods of isolating and concentrating the amino acids were

Table 1. Amino Acid and Vitamin Content of Activated Sludge (Hurwitz, 1957)

Amino Acids, per cent (dry basis)		Vitamins μ gm/gm (dry basis)	pgm/gm
Total Protein (N x 6.25)	30-35 per cent	Riboflavin (B_2)	12.7–29.0
		Cobalamine (B_{12})	2.4– 4.0
Arginine	1.04–1.26	Pantothenic acid	4.0–
Cystine	0.18	Niacine	76.4
Histidine	0.41–0.50	Coline	212.
Isoleusine	0.91–2.20	*Pyridorine	1.2
Leusine	1.58–2.03	*Biotin	0.7
Lysine	0.92–1.33	*Inositol	640.
Methionine	0.45–0.65	*Kibi. Vitamin	10,433
Phenylanine	1.20–2.00		
Threonine	1.15–2.20		
Tryptophane	0.22–0.34		
Valine	1.18–2.77		
Tyrosine	0.70		
Glycine	1.55–1.71		
Glutamic acid	2.89		

Ions and Metals

Common ions	per cent (dry basis)	Trace Metals (Spectrometric Analysis)	
Aluminum	3 -4 per cent	Boron	0.002 per cent
Calcium	2 -3 " "	Chromium	0.1 " "
Iron	5 -6 " "	Copper	0.1 " "
Magnesium	0.5-1 " "	Lead	0.1 " "
Silicon	5 -6 " "	Manganese	0.1 " "
Sulfate (SO_4)	1 " "	Nickel	0.1 " "
Phosphate (P_2O_5)	5 -6 " "	Potassium	0.1 " "
		Silver	0.1 " "
		Tin	0.1 " "
		Titanium	0.1 " "
		Zinc	0.1 " "
		Cobalt	8-15 ppm

Table 2. Analysis of Municipal Activated Sludge (Pillai *et al.*, 1967)

(The results, except vitamin B_{12}, are expressed as percentage on oven dry basis).

Organic matter (loss on ignition)	67.5
Nitrogen (N)	6.0
Crude protein	37.5
Fatty matter (Petroleum ether extractable)	6.0
Crude fibre	9.6
Mineral matter (residue on ignition)	32.2
Silica (SiO_2)	8.6
Calcium (Ca)	1.54
Phosphorus (P)	1.29
Vitamin B_{12}:	
Total activity µg/100 g	76.8
True activity (alkali labile) µg/100 g	73.5

Table 3. Amino Acid Analysis of Organic Molasses (Bouthilet and Dean, 1970)

Amino Acid	Percent dry basis
Arginine	0.79
Cystine	0.17
Glycine	1.88
Histidine	0.37
Isoleucine	1.08
Leucine	1.01
Lysine	0.87
Methionine	0.36
Phenylalanine	0.74
Threonine	1.13
Valine	1.37
Crude protein (Nx6.25)	20

Table 4. Amino Acid and Mineral Content of Activated Sludge From Urbana Waste Treatment Plant

Amino Acids [a]	Percent dry matter	Minerals [b]	Percent dry matter
Arginine	1.20	Ash	32.12
Histidine	0.56	Calcium	3.36
Isoleusine	0.93	Iron	0.80
Leusine	1.89	Magnesium	1.32
Lysine	1.37	Phosphorus	3.24
Methionine	0.48		
Threonine	1.35		

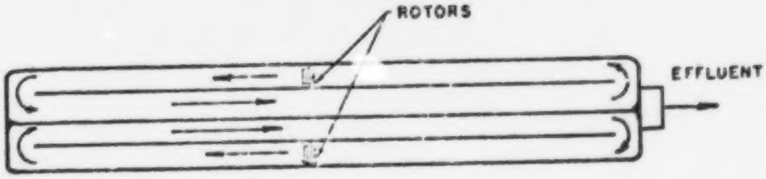
a. Average of two grab samples, 1971.
b. Grab sample, 1974.

reported by Holmes et al., (1971). These studies verified that the major portion of protein was in the very small-sized particles and could be passes through a 200-mesh sieve (74 micron openings), supporting the theory that it is monocellular protein produced by microbial enhancement, Table 5. Screening was not a practical method of concentrating the high proteinaceous fraction of ODML, but the size-analysis studies were of interest because they showed a definite relation between particle size and protein content-the smaller the size, the higher the protein.

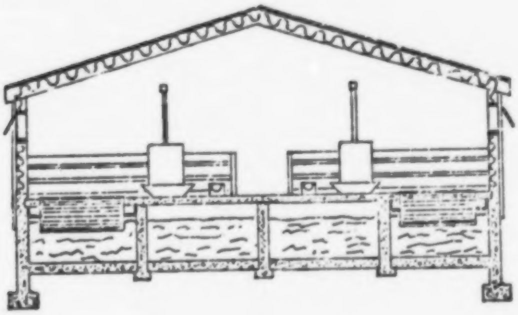
Other methods of concentrating the proteinaceous fraction were also tested, including settling and centrifugation. Using a centrifuge was the most effective method. An amino-acid analysis of swine ODML centrifuge cake is given in Table 6. The protein concentration varied with the depth into the centrifuge cake, the farther into the cake, the more the G force, and the less the amino-acid content. Clarified ODML centrate was also analyzed for its amino-acid content, but none was detected.

Most of the nutrition attention has been given to amino-acid content, partly because amino acids are the most obvious constituent of the single cells and partly because they are the costliest of the nutrients needed to supplement corn. Lysine is one of the most used indicators of amino acid quality because of the low lysine in corn. ODML also contains vitamins and minerals, but these have less economic value than the amino acids at the present time.

The nutritional value of swine ODML was first tested by including a dried product in the diets of growing rats to provide the protein of up to half the soybean meal. Satisfactory gains and efficiency values



PLAN VIEW OF AN OXIDATION DITCH



ELEVATION VIEW

Figure 1 Totally Slotted Swine-Confinement Building With an Oxidation Ditch Beneath the Self-Cleaning Slotted Floors.

resulted (Harmon et al., 1972). Next, finishing swine were used in trials where several ODML refeeding schemes were tested (Day and Harmon, 1972). Two of the simpler schemes are depicted in Figures 2 and 3.

As shown in Figure 2, ODML was pumped from the oxidation ditch into a holding tank where it was further aerated between feedings in order to avoid the possibility of refeeding any unprocessed wastes. To verify the nutritional value, ODML from the holding tank or water was mixed with a 12-percent protein diet in a ratio of two parts liquid to one part feed (approximately the normal ratio of water to feed that finishing swine consume). Regular waterers were provided in all pens. Nutrient composition of the ODML for one experiment is given in Table 7. Corresponding oxidation ditch operating parameters are given in Table 8. In five replications, a total of 76 finishing hogs were fed twice daily in open troughs. The gain and efficiency values were significantly greater for hogs receiving the ODML, Table 9.

Table 5. Amino Acid Contents of Aerobically Treated Livestock Wastes Compared to Corn, Soybean Meal, Swine Feces, and Pig Requirements

(Percent dry matter)

	Beef ODML[a]	Poultry ODML[b]	Swine ODML[c]	Swine ODML[d]	Swine Feces[e]	Pig Req't.[f]	Corn[g]	Soybean Meal[h]
Arginine	1.85	1.43	3.49	1.73	0.44	0.28	0.46	3.09
Cystine	0.56	0.43	0.51	1.30	---		0.12	0.42
Glycine	1.49	1.69	3.70	2.15	---		---	---
Histidine	1.03	0.80	1.39	0.45	0.14	0.24	0.22	1.00
Isoleusine	1.19	1.40	2.96	1.66	0.52	0.27	0.31	2.21
Leusine	2.28	2.48	4.53	2.91	0.92	0.74	1.04	3.69
Lysine	1.68	1.76	3.46	1.64	0.60	0.79	0.26	2.69
Methionine	0.62	0.69	1.38	1.41	---	0.53	0.18	0.63
Phenylalanine	1.34	1.34	3.58	1.62	0.81	0.58	0.42	2.39
Threonine	1.35	1.54	3.13	1.86	0.53	0.49	0.34	1.93
Trypotophan	---	---	---	---	---	0.13	0.058	0.69
Tyrosine	0.96	1.15	1.96	1.36	---	---	0.36	1.73
Valine	1.72	2.11	3.30	2.26	0.58	0.50	0.45	2.36
Aspartic acid	2.75	3.15	5.35	3.82	---	---	0.61	5.82
Glutamic acid	3.79	3.61	9.56	5.37	---	---	1.61	7.74
Crude protein (Nx6.25)	---	---	---	45.6	---	16	8.69	45.7

a,b Grab samples passed through a 200 mesh screen, 1971.
c Avg. of two grab samples passed through a 200 mesh screen, 1971.
d Grab sample, 1967.
e Fresh swine feces (Gowens, 1966).
f Weenling pig (Becker _et at_., 1966)
g,h (Harmon _et al_., 1969).

Table 6. Amino Acid Analysis of Swine ODML Screened and Centrifuged[a] (Holmes, 1971)

(Percent dry matter)

	Centrifuge Cake (Composite)	Varying Radial Distance Into Cake	Contained on 20 Mesh Screen
Arginine	1.15	2.52 - - - 1.15	0.566
Cystine	0.41		
Glycine	2.07		
Histidine	1.00	1.70 - - - 0.77	0.177
Isoleusine	1.24		
Leusine	2.16		
Lysine	1.78	2.85 - - - 1.70	0.432
Methionine	0.54		
Phenylalanine	1.26		
Threonine	1.71		
Tyrosine	1.15		
Valine	2.14		
Aspartic Acid	3.17		
Glutamic Acid	2.90		

a. Sharpes Mark III Centrifuge

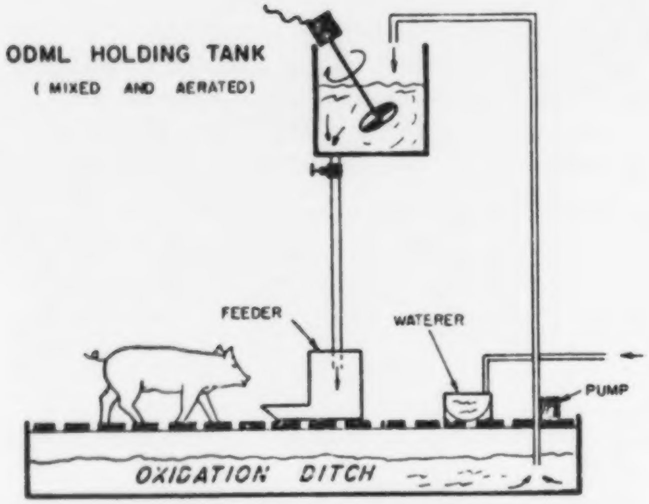

ODML HOLDING TANK
(MIXED AND AERATED)
FEEDER
WATERER
PUMP
OXIDATION DITCH

Figure 2 ODML is pumped from the oxidation ditch into a holding tank where it is kept mixed and aerated between feedings to prevent the possibility of feeding any unprocessed wastes. The ODML is fed by adding it to a regular ration in the ratio of 2 parts ODML to 1 part dry diet. Regular water is provided. (Day and Harmon, 1972).

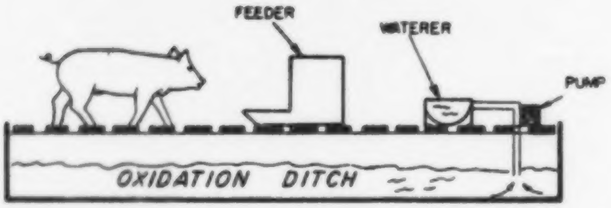

FEEDER
WATERER
PUMP
OXIDATION DITCH

Figure 3 ODML is pumped from the oxidation ditch directly into a watering trough. No other water is provided. (Day and Harmon 1972).

Table 7. Nutrient Content of Swine ODML April-August, 1971
[The oxidation ditch had been in operation
since spring, 1969, without emptying.]

	Percent dry matter[a/]		Percent dry matter[b/]
Phenylalanine	1.48	Calcium	3.33
Lysine	1.42	Phosphorus	3.83
Histidine	.47	Magnesium	1.49
Arginine	1.28	Sodium	2.75
Threonine	1.96	Potassium	4.14
Valine	2.06	Iron	.5507
Methionine	.77	Copper	.0071
Isoleucine	1.49	Zinc	.1148
Leucine	2.79		
Aspartic	3.73		
Serine	2.55		
Glutamic	5.06		
Proline	1.29		
Glycine	2.29		
Alanine	2.83		
Tyrosing	1.17		
Tryptophan	0.28		

a/ Means of 13 weekly analyses of amino acids except for tryptophan (1 analysis).
b/ Mean of 6 weekly analyses of minerals.

Table 8. Summary of Weekly Samples, April-August, 1971
[The oxidation ditch had been in operation
since spring, 1969, without emptying.]

Parameter	Mean	Range
pH	7.7	6.0 - 8.0
Dissolved oxygen, Mg./l.	1.3	0.3 - 4.3
Temperature, °C	27	17.5 - 37.0
Chemical oxygen demand, mg./l.	29,423	18,425 - 55,300
Dry matter, pct.	3.4	2.1 - 4.0
Nitrogen, pct. dry matter	7.9	5.1 - 10.0
Ash, pct. dry matter	41.7	36.1 - 48.7

Table 9. Performance of 76 Finishing Swine Fed ODML or Water Mixed with Feed (Harmon et al., 1973A)

	Water[b/]	ODML[b/]
	pounds	
Average daily gain		
Replication		
1	1.03	1.12
2	1.01	1.21
3	1.08	1.25
4	1.41	1.43
5	1.10	1.17
Average	1.14	1.23
Gain per 1,000 pounds of feed		
Replication		
1	218	232
2	213	249
3	275	276
4	283	302
5	256	270
Average	249	266

Source: Harmon *et al.*, 1973.

[a/] Initial weight, approximately 110 pounds.

[b/] Liquid added to feed, 2:1 ratio.

Table 10. Performance of 120 Finishing Swine Receiving ODML or Water (Harmon et al., 1973)

	Daily gain		Gain per 1,000 pounds of feed	
	Tap H_2O	ODML	Tap H_2O	ODML
		pounds		
Replication				
1	1.34	1.39	303	308
2	1.45	1.54	296	297
3	1.69	1.98	244	343
4	1.32	1.58	230	278
5	1.28	1.45	260	263
6	1.56	1.74	309	338
Average	1.45	1.61	274	304

Source: Harmon *et al.*, 1973. Each value represents 10 pigs.

Hogs slaughtered from each replication showed no evidence of liver or lymphatic alteration due to feeding the single-cell protein source (Harmon et al., 1973A).

As shown in Figure 3, ODML was pumped directly from the oxidation ditch into troughs for 20 seconds out of every 10 minutes. The initial flow flushed out the remaining liquid in the trough, while the flow as the pump stopped remained in the trough. This provided fresh ODML at all times. No other waterers were in the pens. Control swine had access to regular water at all times. A corn-soybean meal diet (12-percent protein) was available in self-feeders in each pen. For three experiments (two replications per experiment) with 120 finishing hogs, the weight-gain and feed-efficiency values were greater for the hogs receiving the ODML, Table 10. (Harmon et al., 1973).

The scheme of Figure 3 uses the processed waste in situ to furnish water requirements as well as other nutrients. With this scheme, there is little if any surplus material for disposal. The levels of non-biodegradable materials will build up, and eventually have to be removed.

A brief energetic and economic analysis of the operation depicted in Figure 3 is given in Table 11. The energetics show a slight loss and the economics show a considerable gain based on the current price of soybean meal. This analysis does not include expenses of initial equipment and maintenance nor does it include savings from other nutrients and reduced disposal.

Table 11. Energetic and Economic Analysis of Feeding ODML to Swine[a]

Aeration Expenses[b]			Protein Savings[e]		Net	
KWH[c]	K Cal	$[d]	K Cal[f]	$[g]	K Cal	$
19.8	17,050	0.40	16,920	0.80	- 130	+0.40
Other expenses:			Other savings:			
Initial equipment Maintenance and repair			minerals Vitamins Water Reduced disposal Odor control			

a Finishing phase of production, 100 to 200 lb. in 60 days.
b Operating expenses only.
c Electricity requirement, 0.33 KWH/Day (p.38 Jones et al., 1971).
d Electricity at $0.02/KWH.
e Based on the ODML supplying 1/5 of the 40 lb. of soybean meal.
f Gross energy of soybean meal, 4.66 K Cal/Gm (Diggs et al., 1965).
g Soybean meal at $200.00/Ton

Studies comparing samples of pork chops and roasts from hogs fed ODML and from control hogs showed that the taste and odor were not influenced by feeding the aerobically sustained product (Wax et al., 1972). Representative samples of all tests have passed state meat inspection in Illinois.

There have, however, been some health problems and some unexpected pig deaths. High nitrate levels in the ODML were suspected of causing deaths in one experiment in which the mixed liquor had been aerated for a considerable time without any hogs in the building. High nitrate levels would be expected under this condition (no new feed for the microorganisms). Another problem has been the survival of intestinal worm eggs that are excreted into the oxidation ditch and are refed in the ODML. No simple method of killing the worm eggs in the ODML has been found. The problems of killing the parasites in biologically treated sewage was discussed by Liebmann (1964). Heating to a temperature of 144F. for a short time was the most successful method of killing worm eggs.

Other studies are underway at the University of Illinois to test the nutrition of various forms of ODML for feeding to poultry and cattle, as well as to swine. A new building for 200 beef cattle with an oxidation ditch beneath slotted floors is now available (Bauling et al., 1973).

SOME OTHER PROJECTS UTILIZING AEROBICALLY TREATED LIVESTOCK WASTES

Vetter et al. (1972) reported the use of beef cattle ODML in situ in the diets for beef cattle. The results of that study suggest that aerobically processed livestock wastes have an acceptable nutritional value and can be effectively used as a partial protein and mineral supplement. No animal health problems associated with feeding the processed wastes or with meat quality were reported.

Studies at Purdue University have shown that aerobic micro-organisms can convert the soluble organic matter in dairy cattle wastes into a biomass containing 30 percent crude protein, Nye et al. (1972). This biomass product was harvested and fed to laboratory rats for up to 20 percent of their diet with no dilatory effect. However, the rats could not use the product as their only protein supplement.

Orr et al. (1972) reported amino-acid contents of swine ODML similar to those in the University of Illinois studies. They also reported of various schemes for refeeding the ODML from oxidation ditches that had been in continuous operation for two years.

Diesch et al. (1971) reported a study at the University of Minnesota for detecting and measuring the survival time of leptospires in aerated beef cattle manure, using a model oxidation ditch. A maximum survival time of 18 days was measured at a pH of 6.9.

SUMMARY

The aerobic process of treating organic wastes produces a biomass with a monocellular-type protein high in amino acids. This makes it of interest as a protein supplement for animal diets that have a major proportion of corn or other grain which is deficient in lysine and other essential amino acids. The biomass also contains other nutrients, including vitamins, minerals, and unidentified growth factors. The health-related aspects of utilizing aerobically processed wastes are of concern, but the aerobic method inherently retards pathogenic organisms. The aerobic process is also a low-odor method of waste management that is of interest to livestock producers. However, the amount of energy required for aeration may be discouraging.

This paper reviews some of the major projects of analyzing the amino-acid content of aerobically treated sewage and livestock wastes and of evaluating the product as a protein supplement in the diets of livestock. Although the amino-acid content is similar for aerobically treated municipal wastes and livestock wastes, extraneous materials can be more closely controlled in livestock wastes than in municipal sewage.

A method developed at the University of Illinois in recent years utilizes oxidation-ditch mixed liquor (ODML) in situ, supplying drinking water as well as protein and other nutrients. Crude protein in the ODML varies from 30 to 46 percent, the latter value is as high as in soybean meal. Also, lysine and other amino acids essential to growth can be as high in concentrated ODML as in soybean meal. This method avoids the ordinary expenses generally associated with recycling. It also offers two obvious advantages: minimizing pollution and realizing a new source of nutrients. The present costs of soybean meal make the method economically feasible and energetically attractive. However, a more efficient method of oxidation is needed. Even so, the aerobic process offers possibilities for a least-cost method of waste management that has several advantages over alternate methods. Obviously, the acceptance of the use of this monocellular protein product in the diets of livestock will require some explanation and education.

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Grass Filtration for Final Treatment of Wastewater*

by

R. M. Butler, J. V. Husted and J. N. Walter**

INTRODUCTION

Grass filtration is one of three systems for land treatment of wastewater[1,2,3]. The other land treatment systems, spray irrigation and rapid infiltration, utilize the plant root zone and the soil profile as a filter medium to renovate wastewater. Grass filtration wastewater treatment systems are designed to utilize the soil surface and plant cover as the filter medium. The treatment mechanisms in the grass filtration system may include physical removal of particulate matter from the water, chemical adsorption of ions in solution on the surface of soil particles, uptake of nutrients in solution by plant roots, and biological treatment by microorganisms. In grass filtration systems, wastewater is applied along the top of a sloping site and flows through the soil-plant filter with subsequent runoff. The runoff from the grass filtration systems can be returned to a stream, held in a pond or reservoir for reuse, or used for ground water recharge. The quality of the runoff water must be compatable with the quality standards of the receiving stream. The runoff water quality depends on the nature of the wastewater, the characteristics of the site, the type of vegetation, and the operating methods of the system.

ADVANTAGES

Grass filtration systems require less extensive wastewater piping systems and less land area than spray irrigation systems. If adequate renovation can be attained by grass filtration, savings in terms of wastewater application equipment and land can be obtained. If treatment by overland flow is adequate, land with low infiltration capacities can be used for wastewater treatment. An additional advantage of the

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grass filtration system is that the treated water remains on the soil surface. This facilitates sampling and monitoring of treatment effectiveness, and the treated effluent is readily available for recycling or reuse.

EXAMPLES

The grass filtration system has been successfully used to treat food industry wastewater. A comprehensive study of an overland flow system for cannery wastewater treatment was conducted for the Campbell Soup Company at Paris, Texas[4]. The results of the study indicate 92 to 99 percent removal of volatile solids, 86 to 93 percent removal of nitrogen and 50 to 60 percent removal or phosphorus. Preliminary tests of a grass filtration treatment system for beef feedlot runoff water have been successful[5].

A spray-runoff treatment system for raw municipal sewage is being tested by scientists at the Robert S. Kerr Environmental Research Laboratory[6]. The experimental system produced an effluent that is of tertiary treatment quality without sludge production.

Only limited information is available on the feasibility of using grass filtration for final treatment of municipal effluent. In a study conducted by Wilson and Lehman[7], municipal sewage effluent from an oxidation pond was applied to grassed plots 1000 feet long for final polishing prior to artifical recharge. During one trial, nitrogen and phosphate concentrations were reduced by approximately 4 and 6 percent, respectively, during flow over the plot. They concluded from these tests that the quality of the effluent obtained from their systems was not suitable for recharge.

PROJECT OBJECTIVES

The primary purpose of the research, summarized in this paper, was to determine if grass filtration can be used to remove nitrate and phosphate from secondary treated municipal sewage effluent. In addition, the effects of various hydrologic parameters on the treatment process were investigated. Factors studied in field and laboratory tests included; detention time, flow rate, flow distance, application frequency, and seasonal effects.

PROCEDURE

Two sets of field tests and a laboratory study were conducted. In the field studies, the effects of hydrologic variables and application frequency on nitrate and phosphate removal were investigated. The effects of detention time on nitrate removal were investigated in the laboratory.

HYDROLOGIC STUDY

Three plots 150 feet long by 20 feet wide were established on a 6 percent slope in the Summer of 1972. A stand of reed canarygrass was established and maintained on the plot area. Stations were installed at 50-foot intervals on each plot to intercept the overland flow for water quality samples and to monitor the flow rate. At each station runoff was intercepted in 4-inch deep plastic lined trenches and directed to an "H.S." flume equipped with a water level recorder to give a continuous record of flow rate. Runoff water quality samples were taken periodically from the outfall of the flume. Three-inch diameter aluminum pipes perforated with 3/4-inch diameter holes spaced 6 inches on center were used to apply the water at the top of each plot and to redistribute the effluent below the monitoring stations 50 and 100 feet down the plot. Water meters were used to monitor the amount of water applied to each plot.

The chemical composition of the municipal effluent illustrated in Table 1 is based on samples collected during 1971 and reported by Sopper and Kardos[8]. The effluent is from the treatment plant which serves The Pennsylvania State University and the borough of State College, Pennsylvania. Secondary treatment includes standard and highrate trickling filters and a modified activated sludge process followed by final settling.

Secondary treated municipal effluent was applied to the plots once each week for six to eight consecutive weeks during the Winter, Spring, Summer, and Fall of 1973. Three wastewater application rates were compared. The same application rate was applied to a plot during the entire study. In the Winter of 1973, 10, 20, and 40 gpm were applied for 8 hours each week. The results of the winter runs indicated that the infiltration capacity of the soil was higher than anticipated. For subsequent trials, the application rates were increased to 15, 30 and 60 gpm and the operating time reduced to 6 hours.

Water samples were taken from the inflow and from the outfall of each flume at 1 1/2 to 2-hour intervals during each run. The nitrate and phosphate concentrations and the pH were determined for all samples. Total Kjeldahl nitrogen determinations were made on selected samples.

Table 1. Chemical composition of the secondary treated municipal sewage effluent used in the study.

Constituent	Minimum	Maximum	Average
pH	7.4	8.9	8.1
	mg/l	mg/l	mg/l
Nitrate-N	2.6	17.5	8.6
Organic-N	0.0	7.0	2.4
NH_4-N	0.0	5.0	0.9
Phosphorus	0.250	4.750	2.651
Calcium	23.1	27.8	25.2
Magnesium	9.1	15.1	12.9
Sodium	18.8	35.9	28.1
Boron	0.14	0.27	0.21
Manganese	0.01	0.04	0.02

APPLICATION FREQUENCY STUDY

Three parallel plots, each 20 feet wide by 150 feet long were installed on a grassed slope. The plots had an uneven slope of 6 to 8 percent, with the greater slope occurring near the lower end of each plot. Each plot was seeded with a mixture of 3 parts of a commercial quick cover seed mixture, 2 parts annual ryegrass, and 2 parts reed canarygrass. The faster germinating ryegrass dominated the growth on each plot during all runs. Effluent was distributed at the head of each plot with perforated pipes similar to those used in the hydraulic study. Effluent collection and monitoring stations similar to those used on the hydrologic study plots were installed at the bottom of each plot.

Three run sequences were conducted during the period from mid-June to early November, 1973. During the first run sequence from June 13 to July 20, effluent was applied to each plot at an approximate rate of 100 gpm. The second run sequence was a continuous flow study in which effluent was applied 24 hours a day for 70 hours at a rate of 40-50 gpm. This run was conducted from August 13-16 on the plot which had received effluent 2 times per week in the first run sequence. A third run sequence, similar to the first, was conducted from September 17 through November 2 with an effluent inflow rate of 50 gpm.

Application frequencies during the first and third run sequences were 2, 3, and 6 times per week with durations of 3, 2, and 1 hours per application, respectively. Each plot received approximately the same total amount of effluent per week. The same application frequency was used on a plot during an entire run sequence.

During the first few weeks of operation, the grass crop was in the development stage. Grass height was maintained at 4 to 8 inches by weekly or semi-weekly cuttings throughout the study. A rotary mower was used and clippings were retained on the plot.

Samples of inflow and outflow were taken for all runs. During certain runs, surface water samples were taken at five additional locations, spaced 25 feet apart, down the plot. Water quality analyses conducted on all samples included nitrate, phosphate, and pH. Total Kjeldahl nitrogen determinations were made on selected samples.

LABORATORY STUDY

A laboratory study was conducted to determine the rate of nitrate removal from secondary treated sewage effluent on the surface of reed canarygrass sod. Three treatments were compared in the study: growing sod, dead sod, and bare subsoil. The sod and the subsoil were from the grass filtration field test site. The dead sod was obtained by drying a sample of growing sod for several weeks. Galvanized steel containers 10 inches wide, 30 inches long and 6 inches deep were used. To drain the containers, a 1-inch diameter perforated conduit was inserted across each end and a 1-inch deep bed of crushed limestone was placed on the bottom. Approximately 4 inches of sod or soil was placed on top of the crushed stone.

The experiments were conducted in a controlled environment chamber with a daytime temperature of 80°F and a nighttime temperature of 65°F. The chamber was lighted by fluorescent and incandescent lighting approximately 14 hours per day. To simulate water flowing down a sloping plot, the containers were placed on a frame that oscillated with an amplitude of 1 inch and a frequency of 15 cycles per minute.

Effluent was applied to the treatments until there was 1/2 inch of free water on the surface. Each day the effluent was drained from the containers and more effluent was applied. Samples of the effluent were taken periodically from the surface water layer during the day for nitrate-nitrogen analysis.

RESULTS

HYDROLOGIC STUDY

Nitrate and phosphate were the nutrients studied in greatest detail. Nitrate removal is possible from two sources; plant uptake and denitrification. Results from a detention time study showed that 50-foot plots with application rates of 15, 30 and 60 gpm had detention times of 15, 10 and 8 minutes, respectively. These flow-through times were too short for any significant plant uptake of nitrate during a run.

Tables 2 and 3 present the percentage of nitrate removed from the wastewater for two different methods of calculation. The values in Table 2 come from a material balance in which the weight of the nitrate applied was compared to the weight lost through infiltration and the weight remaining in the runoff water. The result is a value for nitrate removal that is corrected for dilution caused by melting snow and rainfall. Table 3 is simply a comparison of inflow and outflow nitrate concentrations. A comparison of the two tables shows to what extent the melting snow in the winter and the rainfall in the spring affect the reduction in nitrate concentration. The amount of precipitation that occurred during the Summer and Fall runs was insignificant.

The amount of nitrate removal obtained in this study was quite low compared to those reported for other overland flow studies. Information reported by Law, et al[9], provides a possible explaination for the low nitrate removal. It was reported that a continuous flow for 5 days or more was required before reductions in nitrate levels were obtained. Since the system was operated only once a week for a relatively short period of time, it is possible that anaerobic conditions were not available for dentrification. In addition, the short detention times may not have allowed adequate time for nitrate removal. The laboratory study has shown that detention times of several hours are required for a high percentage of removal. Another factor that makes

Table 2. Effect of flow rate on the percent estimated nitrate loss determined by a nitrate balance during four seasons.

SEASON	Flow Rate[a] Low	Flow Rate[a] Medium	Flow Rate[a] High
		Percent	
Winter	8	5	3
Spring	4	2	2
Summer	3	1	4
Fall	0	1	-1 (gain)

[a] Winter rates for Low, Medium and High were 10, 20 and 40 gpm respectively, other seasons had rates of 15, 30 and 60 gpm.

Table 3. Effect of flow rate on the percent change in nitrate concentration during four seasons.

SEASON	Flow Rate[a] Low	Flow Rate[a] Medium	Flow Rate[a] High
Winter[b]	51	54	33
Spring[b]	17	12	13
Summer	7	4	2
Fall	2	1	-1 (gain)

[a] Winter rates for Low, Medium and High were 10, 20 and 40 gpm respectively, other seasons had rates of 15, 30 and 60 gpm.

[b] High reductions in concentration for Winter and Spring resulted from melting snow and rainfall.

this study different from overland flow studies reporting higher nitrate removals was the nature of the wastewater. The wastewater was highly nitrified secondary municipal effluent and contained relatively small amounts of organic matter. Denitrifying bacteria require organic carbon for a source of energy.

Phosphate removal occurs by plant uptake or by adsorption on the soil particles. Since the water was detained on the plots for such a short period of time, plant removal of phosphate should not have been important during a run. Soil adsorption was possible because of the contact of the water with the surface of the soil. The amount of phosphate removed was low with an average of about 17 percent.

A statistical analysis was performed to determine which parameters significantly influenced nutrient removal. Each increase in flow rate resulted in a significant decrease in phosphate removal efficiency. This indicated that contact with the soil surface is important in phosphate removal. Nitrate removal was not significantly changed by changing the flow rate from 15 to 30 gpm, but switching from 30 to 60 gpm resulted in a significant decrease in nitrate removals. The effects of flow rate on nitrate reduction present a further indication that the time spent on the plots was not long enough at the flow rates used in the study.

The distance traveled by the water also was included in the statistical investigations. Significant decreases in the concentrations of both nitrate and phosphate occurred after each 50 feet of flow. This is a further indication that the most important parameter for nutrient removal is detention time which depends on the flow rate and flow distance as well as the percent slope and the characteristics of the vegetation.

The analysis showed differences exist from season to season. These differences were partly caused by dilution from melting snow and rainfall which can vary greatly from year to year. From a one year study, however, it is difficult to assign much importance to the seasonal effects.

Grass yield data (Table 4) show the effects of the different application rates. For the first 50 feet of the plot receiving 60 gpm, the yield was 2.66 T/A (tons/acre which was lower than all areas sampled except the 50 to 100-foot section of the plot receiving 15 gpm. This area received runoff from the first 50 feet of the 15 gpm plot which was about 6 gpm. The maximum yield was 4.06 T/A for the 50 to 100-foot section of the 60 gpm plot. The first 50 feet of the 30 gpm plot had a yield of 3.27 T/A. The amount of infiltration is an important factor in determining grass yield. The first 50 feet of the 60 gpm plot had a lower yield because approximately 15 inches of water entered the plot during each run. The first 50 feet of the 60 gpm plot and the first 50 feet of the 30 gpm plot each had 12 inches of water entering the soil and produced the largest grass yields.

Table 4. Grass yield for two harvests from 50-foot plot sections receiving effluent at three flow rates.

Flow Rate gpm	Distance Ft.	Yield - Tons per acre dry matter Harvest I	II	Total
15	0-50	1.60	1.09	2.69
15	50-100	1.33	0.90	2.23
30	0-50	2.03	1.24	3.27
30	50-100	1.72	0.96	2.68
60	0-50	1.20	1.46	2.66
60	50-100	2.92	1.14	4.06

APPLICATION FREQUENCY STUDY

Results of the application frequency study were analyzed by comparing nutrient reduction and flow rate relationships. First, mean nitrate-N and PO_4-P concentrations were compared for each of the 3 run sequences. Second, the relationships between the rate of inflow and outflow were examined for each run sequence. Both nutrient reduction and flow rate relationships were examined for effects of application frequency.

First Run Sequence

Effluent was applied at 100 gpm to all plots during the first run sequence. Nitrate-N and PO_4-P reduction apparently had some dependence on application frequency. Comparison of mean nutrient concentration for inflow and outflow indicated that greater overall nutrient reduction was achieved on the plot receiving effluent 2-times per week. An 8.5 percent reduction in PO_4-P was observed on the 2-times per week plot while a 1.6 percent reduction occurred on the 6-times per week plot. The lowest PO_4-P and nitrate-N reduction was observed on the plot receiving effluent 6-times per week. Tables 5 and 6 summarize the results of the first run sequence.

Inflow - outflow effluent flow rate relationships for each plot were similar during the first run sequence. Outflow rate was approximately one-third of the inflow rate once the entire plot had been wetted and a steady rate of infiltration was approached. This condition was achieved for most runs approximately 45 minutes after inflow had started. Thus, two-thirds of the effluent applied was lost through infiltration.

Second Run Sequence - Continuous Flow Study

Results of data collected during the continuous flow run indicate that somewhat greater reductions in nutrient concentration were achieved. Comparison of mean nutrient concentrations of inflow and outflow show a 10.5 percent and 4.9 percent reduction for nitrate-N and PO_4-P, respectively. Variation of nutrient removal with time was minimal during the continuous flow run. Table 7 summarizes data from the continuous flow run.

Inflow during the run varied between 40 and 50 gpm due to scheduled irrigation valve changes elsewhere in the effluent distribution system. Outflow during the entire 70-hour run was approximately one-third of inflow which is an indication of the relatively pervious soil with an infiltration capacity of approximately 1 inch per hour.

Third Run Sequence

Inflow was approximately 50 gpm during the third or late summer run sequence. Application frequency effects for nitrate were similar to those observed during the first run sequence and are shown in Tables 8 and 9. The results for phosphate were different. There was an increase

Table 5. Effect of effluent application frequency on mean nitrate reduction during the early summer run sequence.

	Frequency of Application (per week)		
	2	3	6
Samples in mean	24	25	35
Inflow (mg/l)	11.0	11.8	10.1
Outflow (mg/l)	10.8	11.6	10.1
% Reduction	1.8	1.7	0

Table 6. Effect of effluent application frequency on mean phosphate reduction during the early summer run sequence.

	Frequency of Application (per week)		
	2	3	6
Samples in mean	21	23	35
Inflow (mg/l)	5.11	5.03	5.05
Outflow (mg/l)	4.93	4.93	4.97
% Reduction	8.5	2.0	1.6

Table 7. Mean nutrient reduction during the 70-hour mid-summer continuous flow run.

	Nutrient	
	NO_3-N	PO_4-P
Samples in mean	10	10
Inflow (mg/l)	13.3	4.67
Outflow (mg/l)	11.9	4.44
% Reduction	10.5	4.9

Table 8. Effect of effluent application frequency on mean nitrate reduction during the late summer run sequence.

	Frequency of Application (per week)		
	2	3	6
Samples in Mean	34	45	74
Inflow (mg/l)	11.3	12.2	12.1
Outflow (mg/l)	11.0	12.1	12.1
% Reduction	2.7	0.8	0

Table 9. Effect of effluent application frequency on mean phosphate reduction during the late summer run sequence.

	Frequency of Application (per week)		
	2	3	6
Samples in Mean	34	45	74
Inflow (mg/l)	5.27	5.98	6.22
Outflow (mg/l)	5.19	6.06	6.27
% Reduction	1.5	-1.3*	-0.8*

* Minus denotes increase

in PO_4-P concentration on the plots receiving effluent 3 and 6 times per week. This increase may have been caused by washout of soluble phosphorus from the increasingly dense grass clipping layer at the soil surface.

Inflow-outflow effluent flow rate relationships were more varied and possibly frequency dependent during the third run sequence. Outflow rate was approximately one-tenth of inflow rate for the plot receiving effluent 2 times per week, one-quarter of inflow rate for the 3-times per week plot, and one-third for the 6-times per week plot. The variations observed appeared to be the result of antecedent soil moisture conditions since the 2-times per week plot responded with an outflow rate nearly one-third of the inflow rate when run immediately following an all-night rain.

LABORATORY TESTS

During the first two days of the experiment, there were only slight decreases in the nitrate content of the effluent on the dead sod. After the second day, the daily results were relatively consistent. The nitrate concentration of each wastewater sample was converted to a ratio of nitrate concentration of the sample to the nitrate concentration of the effluent applied that day. The initial nitrate concentration ranged from 12 to 14 mg/l nitrate-N. A set of curves for the data for the third through ninth days of the experiment was obtained using an exponential relationship and regression analysis (Figure 1).

The average reductions in nitrate concentration after 8 hours of incubation were 95 and 72 percent for the growing sod and the dead sod, respectively. The difference in these values can probably be accounted for by plant removal of nitrate. The effluent incubated on the bare subsoil increased in nitrate content during each day. This result indicates that there was not enough organic carbon in the effluent to support denitrification.

The results of the laboratory experiment help explain the results of the field experiments. In the field, the flow-through time for the 150-foot long plots was approximately 30 minutes. Under laboratory conditions, a 22 percent reduction in nitrate concentration was obtained on the growing sod in this time. The maximum reduction in nitrate content obtained in the field was approximately 10 percent during the continuous flow test. The difference between this value and the laboratory value for the same detention time is probably due to the fact that the laboratory test was for a batch system and the field tests were for a continuous flow system subject to temperature variations. If under the best field conditions, the removal rate is about one-half that obtained in the laboratory, detention times of about 12 hours would be required in the field to reduce the nitrate concentration from 12 to 1 mg/l NO_3-N. Additional laboratory tests are being conducted to determine the effects of initial nitrate concentration of the effluent and temperature on nitrate removal.

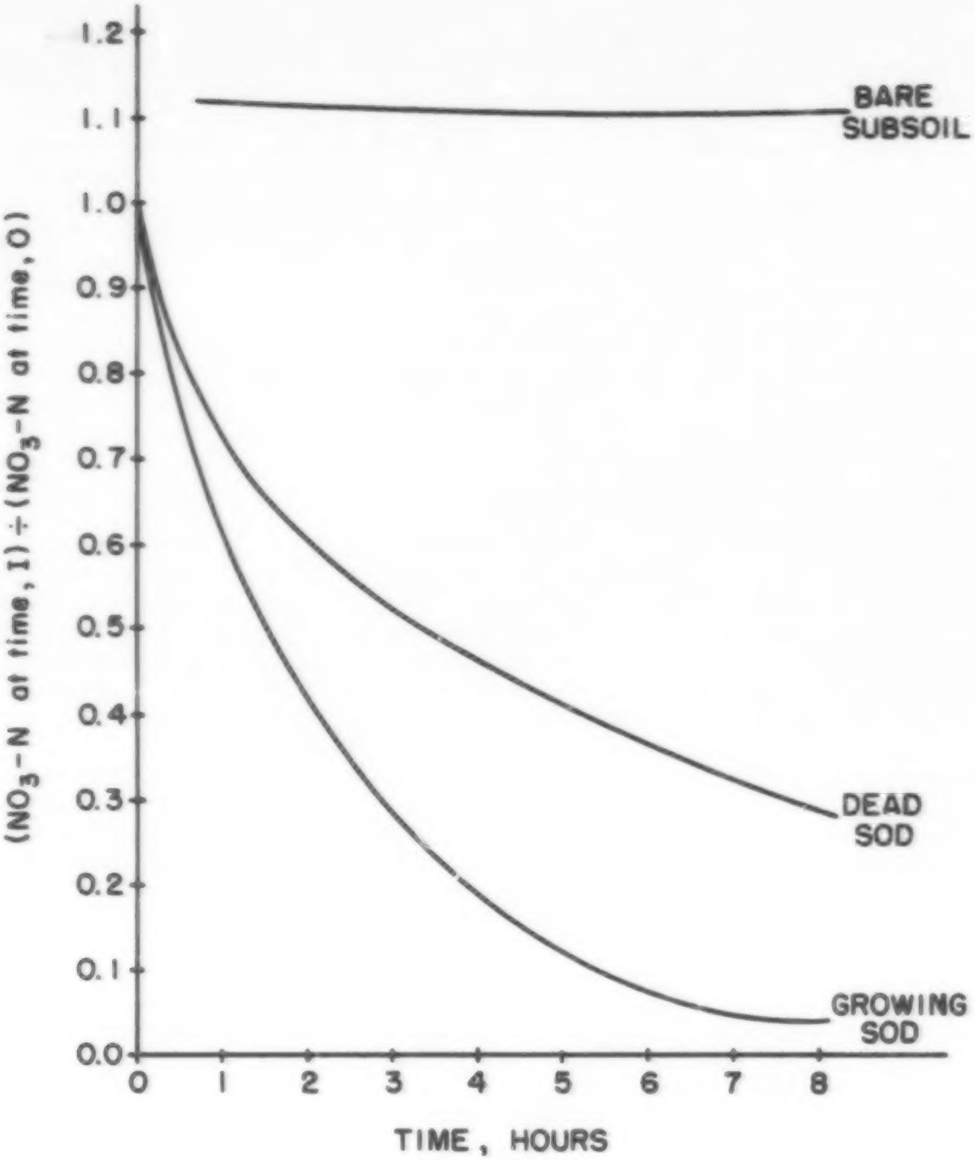

Figure 1. Nitrate removal from wastewater during incubation on three laboratory treatments.

CONCLUSIONS

No significant reduction in phosphate or nitrate concentrations was observed in secondary treated municipal sewage effluent after grass filtration with the flow rates, plot slopes, and plot lengths used in this study. The results of the tests will be useful, however, in planning future experiments in which the application procedure and the plots will be modified to improve nutrient removal.

The results of the field studies indicate that the removal of both phosphate and nitrate increased as the application frequency was decreased from 6 times to 2 times per week. The duration of application was increased as the frequency was reduced so that approximately the same depth of effluent was applied to each plot. Comparison of samples taken during each application period indicated only slight differences in both nitrate and phosphate renovation during a run. These results suggest that the grass filtration system should be operated with runs of long duration separated by several days of rest. The field tests also indicated that leaving grass clippings on the plot surface may result in increased phosphate concentrations as phosphate is leached from the decomposing grass into the wastewater.

The greatest problem to be overcome in designing a grass filtration system to remove relatively high nitrate concentrations from municipal sewage effluent is to obtain sufficiently long detention times. In laboratory tests, 5 to 6 hours were required to achieve a 90 percent reduction in nitrate concentration from effluent with an initial concentration of 12 mg/l NO_3-N. Under field conditions, the detention times would probably have to be 1 1/2 to 2 times the laboratory values for a similar reduction in nitrate concentration. Detention times of this length could be obtained by restricting the flow rate over the plots with barriers or by recycling the effluent.

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USE OF CATTLE FEEDLOT RUNOFF IN CROP PRODUCTION[1]

by

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ABSTRACT

Land disposal of beef-feedlot-lagoon (run-off) water was studied. Lagoon water was applied during the summers of 1970, 1971, 1972 and 1973 by furrow irrigation to a silty clay loam soil. After four years the five treatments averaged 0, 7, 13, 22 and 37 cm/yr. Corn (Zea mays L.) forage yield and plant content of N, P, K, Ca, Mg, and Na were measured. Surface soil samples and soil cores were taken from the plots after harvest each year.

Electrical conductivity ranged from 1.6 to 7.6 (3.1 average) mmho/cm in the lagoon water applied at the study site and from 1.0 to 12.8 mmho/cm in samples taken from 12 Kansas feedlots. Electrical conductivities of extracts from saturated pastes of the surface soil samples were increased linearly by accumulative treatment all years. The 1970, 1971 and 1972 soil cores showed accumulations of NO_3^--N, P, K, and Na in the top 30 cm at all treatment rates. Movement of NO_3^--N and Na down to 100 cm was noted in 1971 in cores from plots receiving 43 cm/yr. Movement of NO_3^--N down to 240 cm was recorded in 1972 in cores from plots that had received 20 and 41 cm/yr. Extractable Ca and Mg in the soil cores was not affected by treatment. Corn forage yields were a linear function of treatment in 1970 and a quadratic function in 1971, 1972 and 1973. The positive effect on yield was attributed to increased soil fertility; the relative decreases at the higher rates were attributed to increased soil salinity. Maximum yield and uptake of N and P were reached at the 13 cm/yr disposal rate in 1971 and 1972, and at the 22 cm/yr rate in 1973.

Key Words: Feedlot waste, feedlot lagoon, waste-water, forage production, irrigation, soil salinity.

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INTRODUCTION

The increasing size of beef cattle feedlots has been paralleled by an increase in the magnitude and incidence of their waste disposal problems. The Kansas State Department of Health requires that runoff detention lagoons hold three inches of surface runoff from feeding areas and that these lagoons be emptied as soon as practical to maintain retention capacity (3). The most common method of disposal has been to apply lagoon water to adjacent crop and pasture land. High transportation costs dictate that maximum rates be applied to these fields close to the source.

Care must be taken so that the soil used as the disposal medium does not become polluted. Characterization of beef-feedlot runoff has shown it to vary widely in chemical composition, and generally to be high in total salt content (1,2,5,6,7,8,9). After runoff arrives at a lagoon, evaporation can further increase salt concentrations. When such lagoon water is applied to a soil, some of the salts can be used as plant nutrients to increase productivity but excess salts can create soil salinity and dispersion problems.

Only limited data are available on effects of beef-feedlot-lagoon water on soil properties and plant growth. Travis, et al. (10), found total salts increased by 200% and infiltration declined to zero in soil columns from four Kansas soils after inundation with lagoon water with an electrical conductivity (EC) of 13.4 mmhos/cm. They suggested that the higher proportion of monovalent cations Na^+, K^+ and NH_4^+ in such soil could have dispersed colloids and led to the cessation of infiltration.

Satterwhite and Gilbertson (8) found in a [illegible]year field study that sprinkler applications of up to 30.5 cm of lagoon water increased soil N, P, K, S, and Cl. They compared (in a greenhouse) the effects of tap water, water taken from a lagoon in 1969, and water from the same lagoon in 1970 on plant growth. Both lagoon waters increased total salts in the soil more than did tap water. Growth of nine grass species was enhanced by tap water and by the 1970 lagoon water, but was inhibited by the 1969 lagoon water.

We evaluated how growth and composition of corn (Zea mays L.) forage and chemical properties of a Kansas soil were affected by furrow irrigation with beef-feedlot-lagoon water.

MATERIALS AND METHODS

The study area was located 6 miles north of Pratt, Kansas, on a silty clay loam soil with a cation exchange capacity of 19 meq/100g and a pH of 7.0. Lagoon water was obtained from a nearby commercial feedlot and was applied by furrow irrigation in increments of 5 and 10 cm during the summers of 1970, 1971, 1972 and 1973 to plots 9 m wide and 61 to 111 m long. Five treatments were replicated four times. In 1970 and 1973 the last 10 cm increment of lagoon water was not applied to the four plots receiving heaviest applications until after harvest. Inflow and outflow was measured on each plot. The average amounts applied after four years were calculated to be 0, 7, 13, 22 and 37 cm per year for the four replications. The two replications that were sampled for soil core analysis had received an average of 0, 8, 17, 26 and 45 cm per year by the fall of 1970, 0, 8, 15, 23 and 43 cm per year by the fall of 1971, and 0, 7, 14, 20 and 41 cm per year by the fall of 1972. Samples of the lagoon water were collected during each application and analyzed for Ca, Mg, Na, and K by standard atomic absorption and flame photometric procedures, for ammonium-nitrogen (NH_4^+-N) and nitrate-nitrogen (NO_3^--N) by steam distillation procedures (4), and for soluble salts by resistance measurements using a Wheatstone bridge.

To compare composition of the material being applied at the study site with that found at other feedlots, samples were taken from 12 locations in Kansas during early July of 1970 and 1971, and were analyzed in the manner described above. The lagoons were sampled at four depths in 1970: 5, 50, 100, and 200 cm. Chemical composition did not vary with depth in 1970, so a single sample was taken at 15 cm from each lagoon in 1971.

All plots received a preplant irrigation in the spring of 1971 and 1972. Additional well water was applied during the growing seasons to the control plots and to plots receiving the lower lagoon-water treatments so all plots received approximately equal volumes of liquid. No chemical fertilizers were applied.

Corn silage yields (mechanical harvest) were recorded, and samples from each plot were analyzed for Ca, Mg, Na, and K by atomic absorption and flame photometry after a nitric-perchloric acid digestion. Nitrogen and P were determined by steam distillation and colorimetric procedures respectively after a sulfuric acid digestion. The 1972 and 1973 forage was analyzed for nitrate-nitrogen by hot-water extraction and subsequent analysis by steam distillation.

Composite surface soil samples (0 to 15 cm) were taken at 18 locations in each plot after harvest. Extracts from a water-saturated paste were analyzed for soluble salts by resistance measurements. Cores were taken after harvest to a depth of 2 m in 1970 and 3 m in 1971 and 1972 from plots in two replications. The surface meter of each core was divided into 10 cm increments and analyzed for extractable Ca, Mg, Na, and K by atomic absorption and flame photometry. For the 1970 and 1971 cores the extraction procedure included an initial extraction with methanol to remove the cations not on exchange sites. This was followed by extraction with 1.0 N ammonium acetate, pH 7.0, to remove the exchangeable cations. The 1972 cores were extracted with ammonium acetate only. Except for Na, only insignificant concentrations were found in the methanol extract. The total cations extracted by these two methods are referred to as extractable Ca, Mg, Na, or K. The surface meter increments also were analyzed for Bray P-1 extractable P. The lower depths were divided into 20 cm increments and, along with the upper depths, analyzed for NO_3^--N by steam distillation techniques (4). The 1972 cores were analyzed for soluble salts to a depth of one meter by resistance measurements of extracts from water-saturated pastes.

RESULTS AND DISCUSSION

Lagoon Water Analyses

Results of analyses of the lagoon water applied are given in Table 1. Total salt concentration, indicated by the electrical conductivity, was high. Concentrations of the monovalent cations NH_4^+, K^+, and Na^+ were considered to be high. The high and low values of all measurements show how the composition of the lagoons of a single feedlot can vary. This variation was not accounted for in our interpretation of soil and plant measurements.

Table 2 includes the analysis of the lagoon samples taken at the 12 Kansas locations. Wide variation in composition of samples among lagoons each year was similar to variation of lagoon water applied. There was little correlation between the 1970 and 1971 data from the same locations. Lack of uniformity between lagoons and between years makes it essential that analysis of lagoon waste water be known before disposal to predict how the material will affect soil properties and plant growth.

Effects On Soil Chemical Properties

Electrical conductivities of saturation extracts from 1970, 1971 and 1972 surface soil samples were linearly related to the accumulative cm of lagoon water applied (Fig. 1). The EC values of plots receiving an average of 7 cm/yr were not much higher than those of the control plots. Plots receiving 13, 22, or 37 cm/yr had EC values generally higher than the control plots. Since the EC measurement is directly related to total water soluble salt content of a soil, the three heaviest treatments provided more salts than could be used by the corn plants or leached lower into the profile. High R^2 values for all three years (0.878, 0.875 and 0.914) indicate good correlation between the EC measurement and accumulative amounts of lagoon water applied. This suggests that EC measurements could be used to estimate amounts of lagoon water that have been applied to a soil.

To improve the graphical presentation of the soil-core analysis data, measurements taken from two plots receiving the same treatment were averaged. Data points were further reduced by combining data from adjacent depth increments, so that in Figs. 2, 3, 4, and 5 values are plotted for depths of 10, 30, 50 cm, etc., rather than at depths of 5, 15, 25, 35, 45, 55 cm, etc.

Fig. 2 gives NO_3^--N concentrations in the 1970, 1971 and 1972 soil cores. In 1970, all plots that received lagoon water had higher NO_3^--N concentrations at the 10 and 30 cm depths than did control plots. No NO_3^--N accumulated deeper than 30 cm in 1970. Soil cores taken in 1971 showed accumulations at 10, 30 and 50 cm depths at all dispersal rates. The 23 and 43 cm/yr rates caused particularly high concentrations at 10 and 30 cm depths. A NO_3^--N peak was found at 100 cm under the plots that received 43 cm/yr. In 1971 peaks were less well defined at 200 and 240 cm under the plots that had received lagoon water treatments of 15 and 43 cm/yr, respectively. By 1972 there was significant movement of NO_3^--N to the 240 cm depth under plots that had received 20 and 41 cm/yr. Because the control plots received no additional N, their NO_3^--N levels were lower than what would be expected in an irrigated soil being managed for maximum corn production. The two highest disposal rates were, therefore, the only treatments that caused significant accumulations of NO_3^--N in the soil profiles.

All disposal rates caused extractable P to accumulate to a depth of 10 cm in the 1970, 1971 and 1972 soil cores (Fig. 3). Movement to the 50 cm depth was found under plots receiving 41 cm/yr in 1972. The general lack of P movement indicates that the soil's capacity to immobilize the added P had not been exceeded except possibly at the highest application rate.

Analyses of the 1970 cores for extractable Na, K, Ca, and Mg, and analyses of 1971 and 1972 cores for extractable Ca and Mg showed no trends due to treatment. Extractable K was increased to a depth of 10 cm in the 1971 cores and to 30 cm in the 1972 cores at all disposal rates (Fig. 4). Movement below these depths was probably restricted by exchange reactions with clay colloids. Extractable Na accumulated at all depths in the 1971 and 1972 cores (Fig. 5). Deeper movement of Na than K reflects greater competitiveness of K for cation exchange sites. Soluble salts as indicated by the EC measurements of the 1972 soil cores had increased throughout the top meter under plots receiving 14, 20 and 41 cm/yr (Fig. 6).

Effect On Yield, Elemental Uptake And NO_3^--N Content Of Corn Forage

Thirty-five cm of lagoon water had been applied to the plots receiving heaviest treatments by the time the 1970 corn forage was harvested. There was a general linear trend in yield due to treatment in 1970 (Fig. 7). That positive effect on yield in 1970 probably resulted from increased soil fertility (nutrients in the lagoon water).

Maximum yields were recorded at the 13 cm/yr rate in 1971 and 1972 and at the 22 cm/yr rate in 1973. Yields fell off at higher application rates, which gave a quadratic relationship between accumulative applications and yield in 1971, 1972 and 1973 (Fig. 7). The initial increase in yield again can be attributed to improved soil fertility, while the yield decline at the higher rates likely resulted from salt buildup in the soil (Fig. 1). Yield depression was less in 1973 probably because of higher rainfall that year. The yield decline was relative; all plots receiving lagoon water yielded more than control plots.

Uptake of N, P, K, Ca, Mg, and Na was calculated from the yield and elemental analyses of the corn forage. Linear and quadratic coefficients and intercepts from a regression analysis on uptake as affected by the accumulative applications of lagoon water are presented in Table 3. In 1970 uptake of all elements increased linearly with applications as did the 1970 yield. Uptake in 1971 and 1972 was a quadratic function of treatment as was yield. Maximum uptake of most elements occurred at about the same application rate that gave the highest yield (Figs. 7, 8, 9 and 10).

The concentration of nitrate-nitrogen increased linearly with accumulative treatment in the 1972 and 1973 corn forage (Fig. 11). Two of the values measured were high enough to be considered dangerous to livestock if ingested.

SUMMARY AND CONCLUSIONS

Feedlot-lagoon water can contain large amounts of salts, particularly the monovalent cations. Considerable variations in composition of feedlot-lagoon water is to be expected with varying rainfall, runoff, and evaporation. Continued applications of feedlot-lagoon water significantly increased salt content of soil we studied. Increases in the electrical conductivity of the soil were linearly related to the amount of lagoon water applied. The heaviest lagoon-water treatments contributed more salts than could be utilized by corn plants or leached into the lower portions of the soil profile. The resultant accumulations could have produced higher osmotic pressures in the soil solution. Nitrate-nitrogen accumulated in the soil from the 22 and 37 cm/yr lagoon water applications reflecting the relatively high nitrogen content of lagoon water. Phosphorus also accumulated with lagoon water applications but accumulations were mostly restricted to the surface 20 cm, reflecting lack of movement of P in the soil. Maximum yields of corn forage occurred at an average application rate of 13 cm/yr after the second and third year and at 22 cm/yr after the fourth year. At higher rates, yields declined. Maximum removal rates of applied nutrients, an important consideration in maintaining viability of soil, were achieved at the same application rates that produced maximum yields.

Disposal of feedlot-lagoon water can produce excess accumulations of salts if soil composition is not closely monitored. Large amounts of monovalent cations may contribute to undesirable conditions by restricting water infiltration by dispersion of soil colloids. Yearly soil analyses of disposal areas are recommended. Results of this investigation suggest that electrical conductivity measurements can be used to estimate the amount of lagoon water previously applied and to predict effects of applications on soil chemical conditions and plant growth.

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Table 1. Elemental Composition And Electrical Conductivity Of Beef-Feedlot-Lagoon Water Applied To Research Plots During 1970, 1971 And 1972.

	Electrical Conductivity	Na	K	Ca	Mg	NH_4^+-N	NO_3^--N	Total Solids	COD	Volatile Solids
	mmho/cm					mg/l				%
High	7.6	660	1840	615	239	179	63	18134	6445	44.4
Low	1.6	112	259	83	35	4	1	1879	593	27.1
Average	3.1	295	671	225	87	73	13	4771	1868	37.8

Table 2. Elemental Composition And Electrical Conductivity Of Beef-Feedlot-Runoff Lagoon Samples Taken At 12 Locations In Kansas During July, 1970 and 1971. The 1970 Data Are Averages Of Samples Taken At Four Depths: 5, 50, 100 and 200 cm. The 1971 Data Are From A Single Sample Taken At 15 cm.

Location	Electrical Conductivity		Na		K		Ca		Mg		Total Inorganic N (NH_4^+-N + NO_3^--N)	
	1970	1971	1970	1971	1970	1971	1970	1971	1970	1971	1970	1971
	mmho/cm		ppm									
1	3.1	3.0	142	122	387	583	152	101	74	78	195	145
2	2.7	2.7	139	220	166	325	101	100	61	70	164	119
3	3.3	4.1	221	293	330	604	160	158	85	111	151	167
4	1.0	4.8	73	329	52	617	65	127	34	124	16	161
5	4.2	3.9	268	335	550	729	141	143	97	81	220	61
6	6.3	4.3	339	299	826	708	177	135	78	89	130	218
7	8.6	12.6	653	1320	1100	1920	523	310	153	171	366	543
8	12.8	4.2	1690	403	2030	458	403	136	189	82	207	127
9	7.3	---*	834	---*	752	---*	212	---*	112	---*	221	---*
10	---*	10.6	---*	1460	---*	2210	---*	108	---*	235	---*	136
11	---*	1.4	---*	317	---*	483	---*	101	---*	64	---*	133
12	---*	6.0	---*	500	---*	750	---*	187	---*	170	---*	143

* Lagoon dry at sampling time.

Table 3. Corn Forage Yield, Electrical Conductivity Of Soil Saturation Extracts (EC), and Uptake By Corn Of Indicated Elements In 1970, 1971, 1972 and 1973 As Affected By Accumulative Applications Of Beef-Feedlot-Lagoon Water (cm). The Data Are Expressed As Factors Of A Regression Equation: $y = ax^2 + bx + c$.

Year	Measurement (y)	R^2	a	b	c
1970	Yield (MT/ha)	0.316	0.0	0.498*	41.5
	N uptake (kg/ha)	0.482	0.0	2.75*	98.9
	P uptake (kg/ha)	0.107	0.0	0.388	33.7
	K uptake (kg/ha)	0.197	0.0	1.60*	162
	Ca uptake (kg/ha)	0.0	---	---	---
	Mg uptake (kg/ha)	0.186	0.0	0.0890	19.8
	Soil EC (mmho/cm)	0.878	0.0	0.0166*	0.495
1971	Yield (MT/ha)	0.372	-0.00410*	0.425*	36.3
	N uptake (kg/ha)	0.574	-0.0221*	2.63*	85.0
	P uptake (kg/ha)	0.290	-0.00256*	0.264*	17.2
	K uptake (kg/ha)	0.079	-0.000190	0.367	131
	Ca uptake (kg/ha)	0.157	-0.00280	0.301	20.9
	Mg uptake (kg/ha)	0.188	-0.00139	0.137	14.8
	Na uptake (kg/ha)	0.387	-0.000212*	0.0261*	0.649
	Soil EC (mmho/cm)	0.875	0.0	0.0191*	0.470
1972	Yield (MT/ha)	0.269	-0.00322*	0.426*	49.2
	N uptake (kg/ha)	0.584	-0.0177*	2.91*	96.3
	P uptake (kg/ha)	0.161	-0.00179	0.270	29.3
	K uptake (kg/ha)	0.465	-0.0183*	2.83*	164
	Ca uptake (kg/ha)	0.212	-0.00237	0.350	28.9
	Mg uptake (kg/ha)	0.132	-0.00117	0.170	21.8
	Na uptake (kg/ha)	0.333	---	0.00415*	0.558
	Soil EC (mmho/cm)	0.914	---	0.0200*	0.391
	Forage Nitrate-Nitrogen (ppm)	0.561	---	5.29*	-8.86
1973	Yield (MT/ha)	0.327	-0.00106	0.245*	33.8
	Forage Nitrate-Nitrogen (ppm)	0.482	---	2.26*	75.7

* Coefficient significant at the 0.05 level.

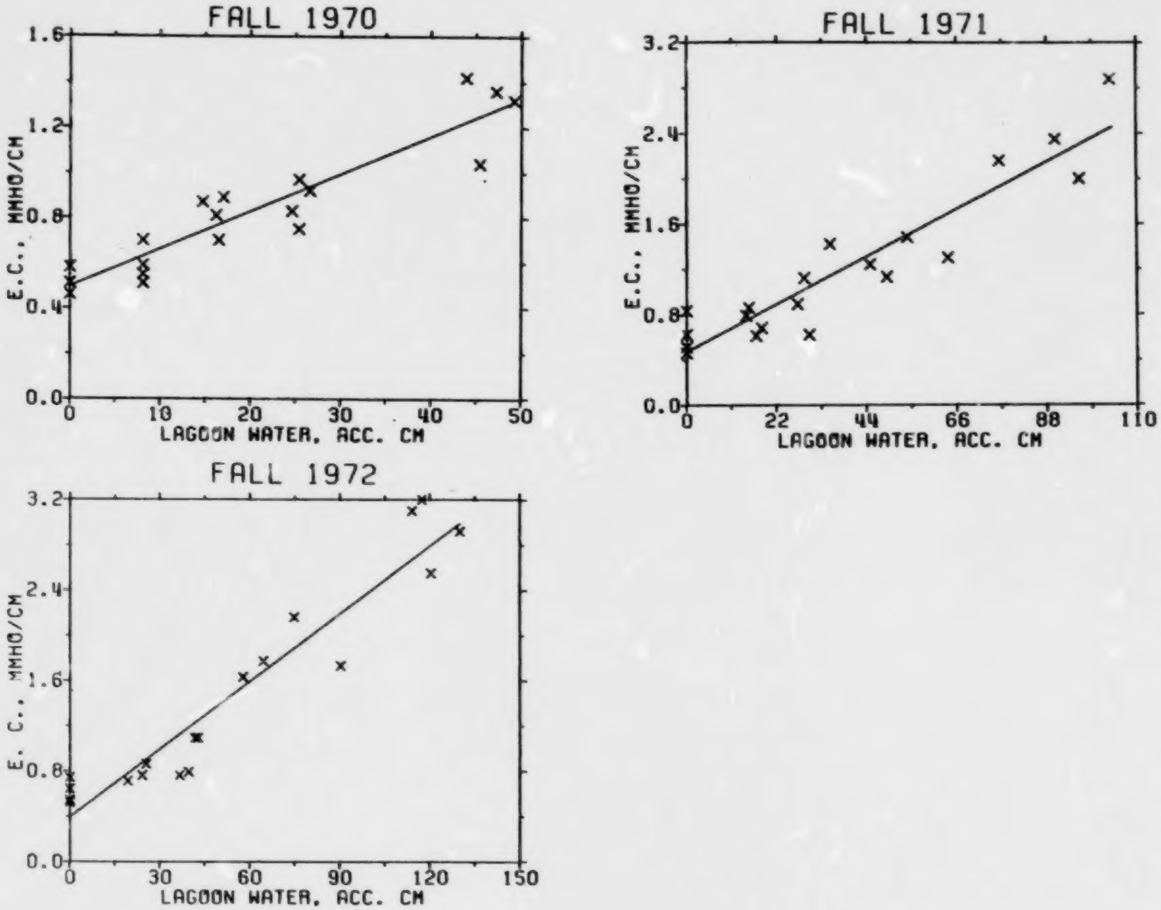

Fig. 1. The electrical conductivity (EC) of saturated paste extracts from surface (0-15 cm) soil samples taken in the fall of 1970, 1971 and 1972 as affected by accumulative applications of beef-feedlot-lagoon water.

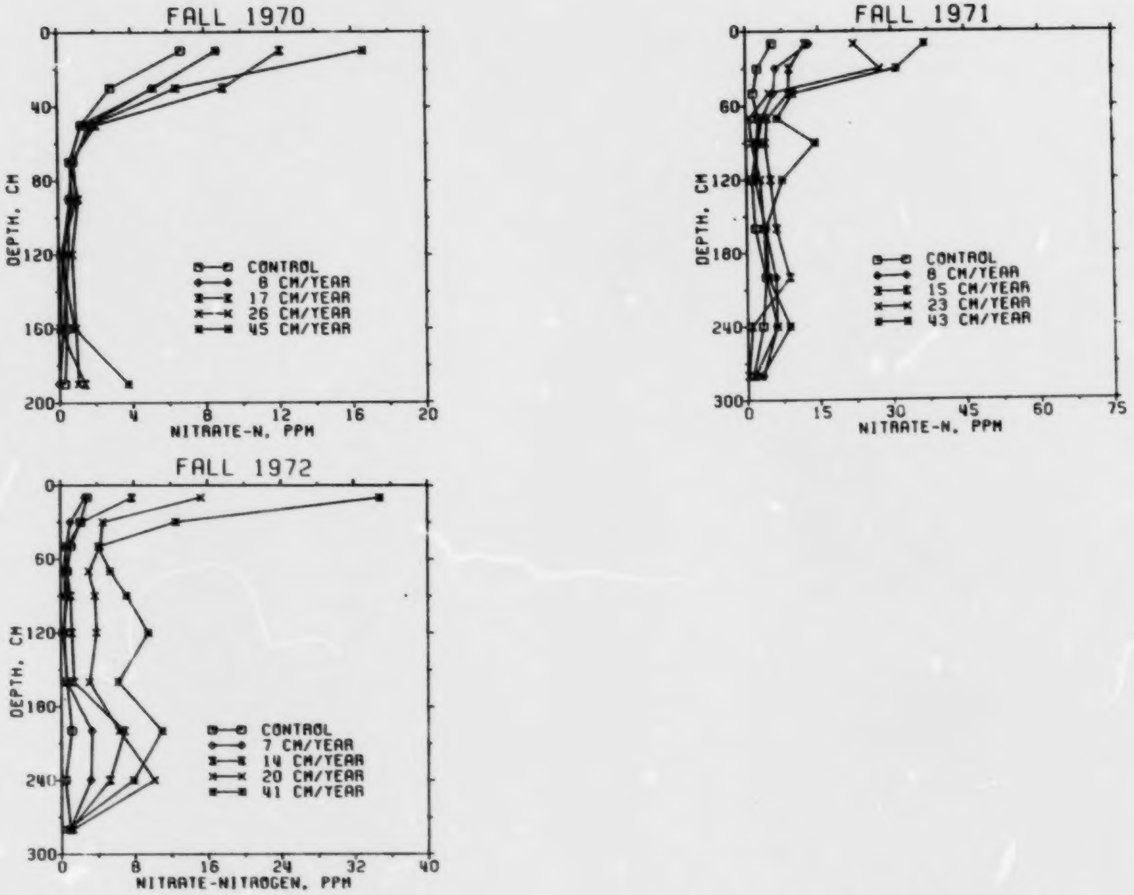

Fig. 2. The NO_3^--N content of soil cores taken in the fall of 1970, 1971 and 1972 as affected by depth and average yearly application rate of beef-feedlot-lagoon water.

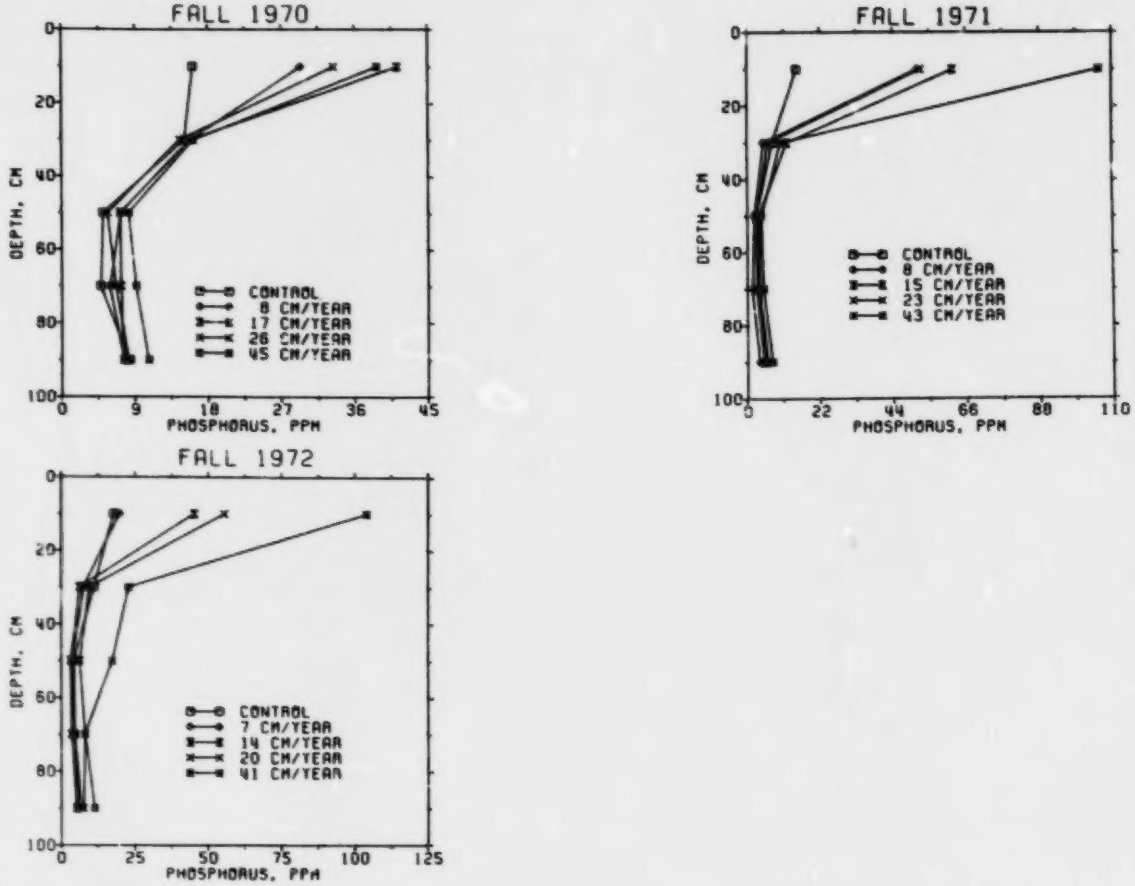

Fig. 3. The Bray P-1 extractable P content of soil cores taken in the fall of 1970, 1971 and 1972 as affected by depth and average yearly application rate of beef-feedlot-lagoon water.

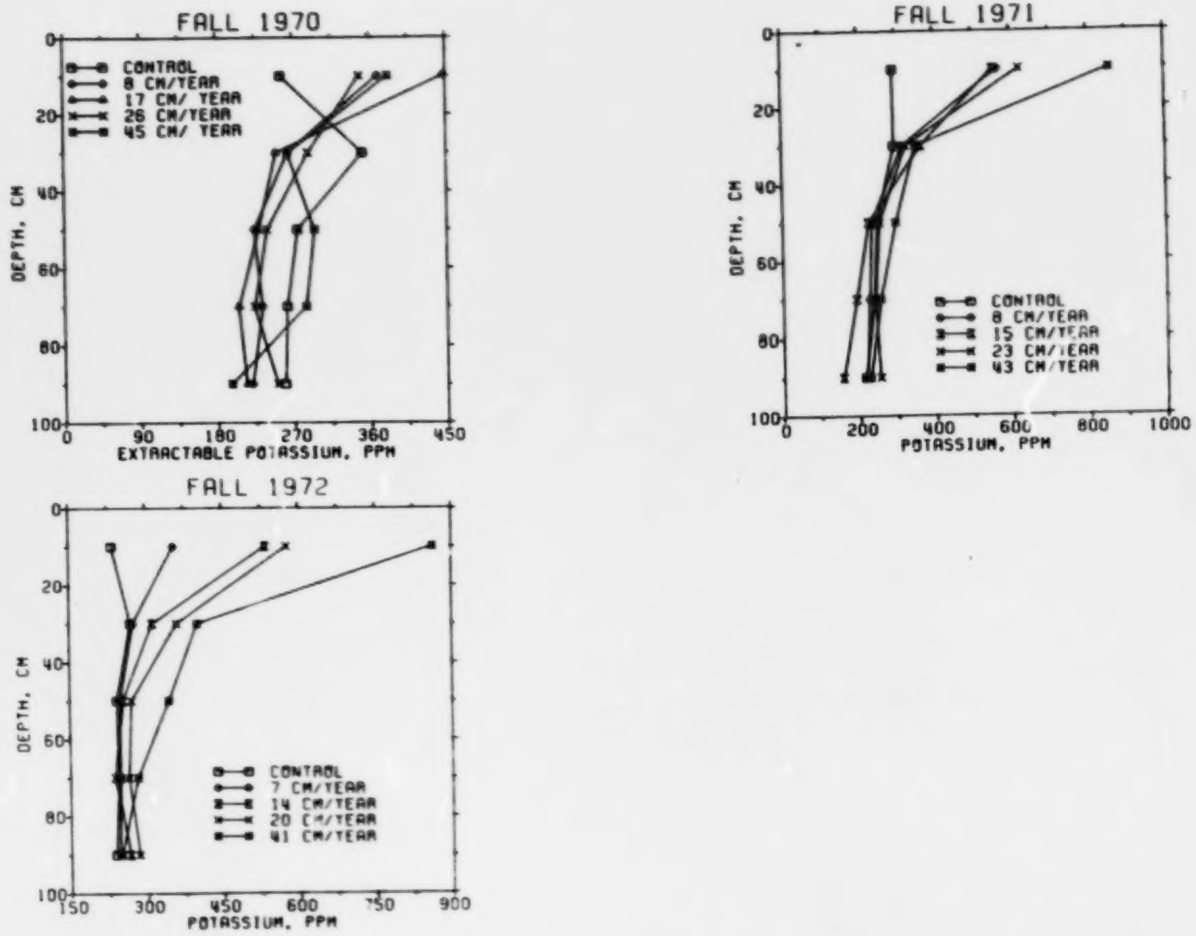

Fig. 4. Extractable K content of soil cores taken in the fall of 1970, 1971 and 1972 as affected by depth and average yearly application rate of beef-feedlot-lagoon water.

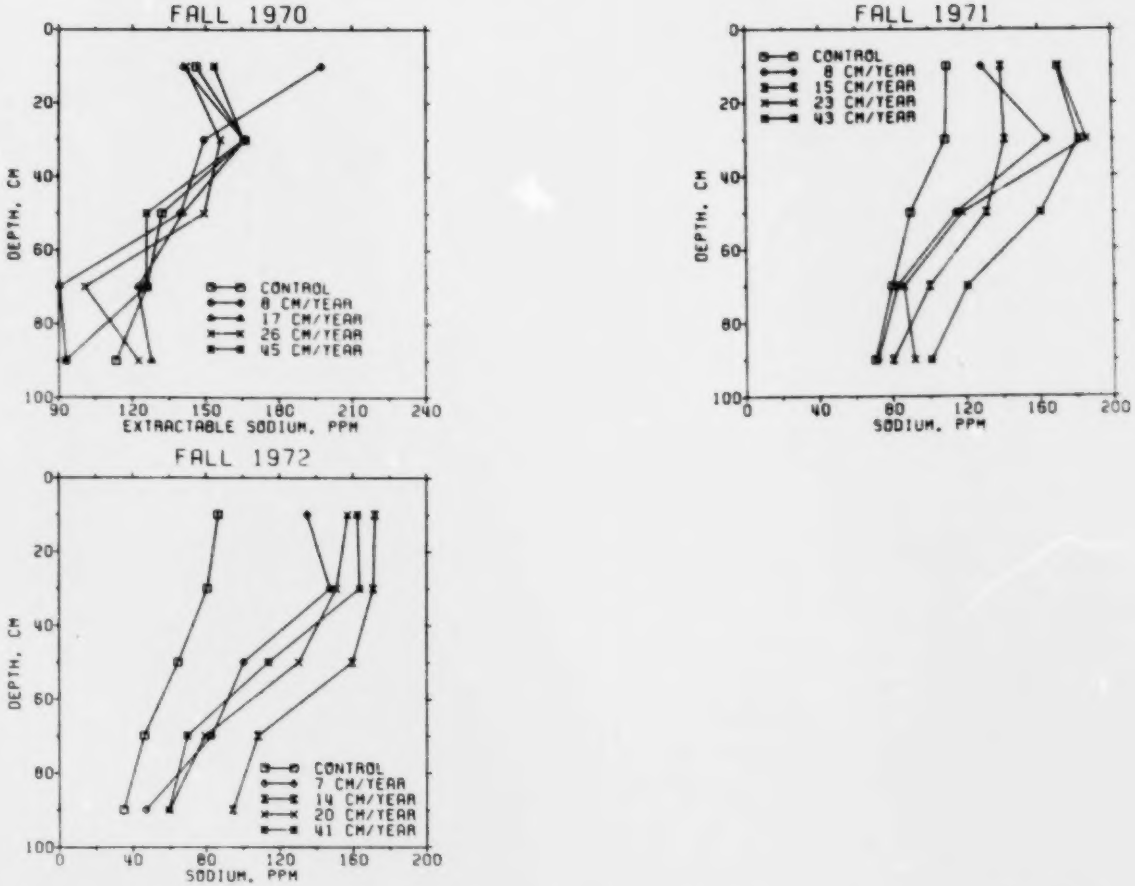

Fig. 5. Extractable Na content of soil cores taken in the fall of 1970, 1971 and 1972 as affected by depth and average yearly application rate of beef-feedlot-lagoon water.

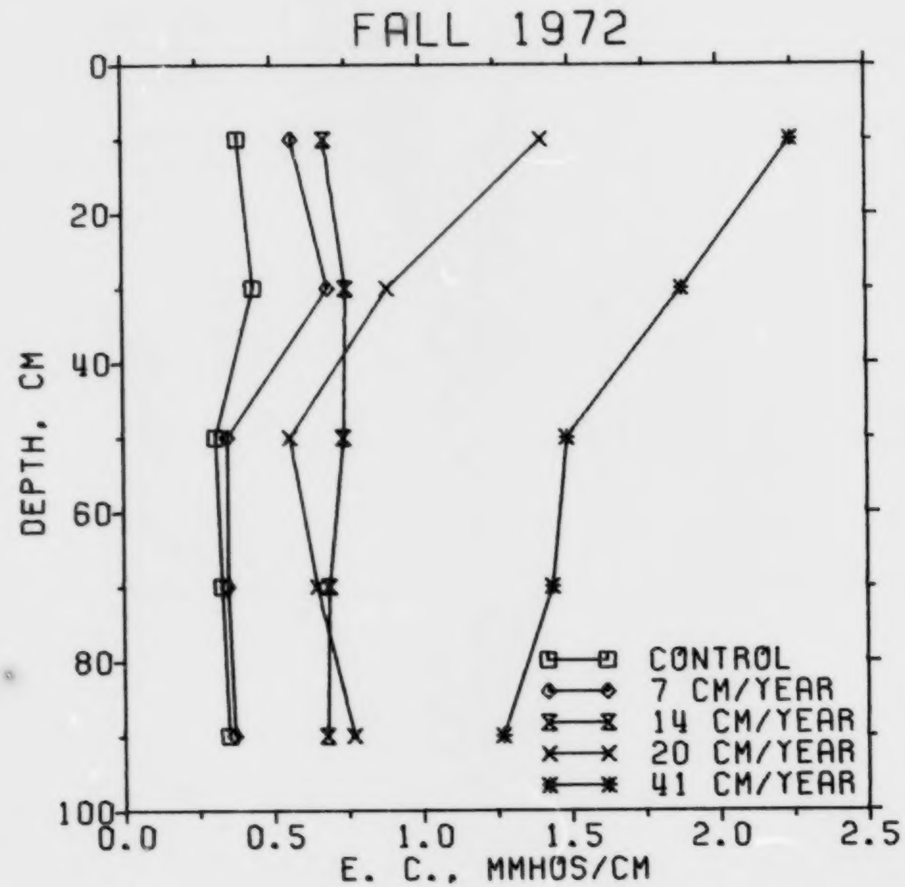

Fig. 6. The electrical conductivity of saturated paste extracts from soil cores taken in the fall of 1972 as affected by depth and average yearly application rate of beef-feedlot-lagoon water.

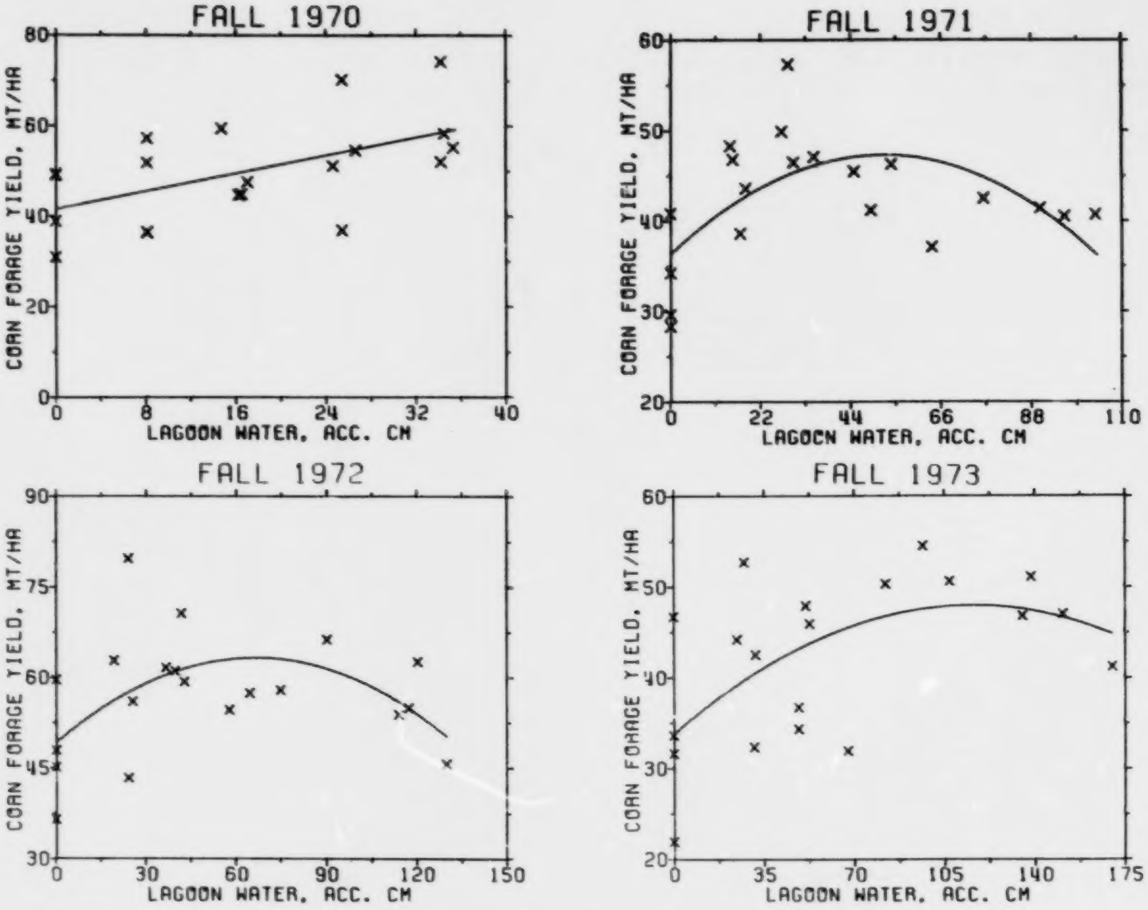

Fig. 7. Corn forage yield (metric tons/hectare, corrected to 30% dry matter) in 1970, 1971, 1972 and 1973 as affected by accumulative applications of beef-feedlot-lagoon water.

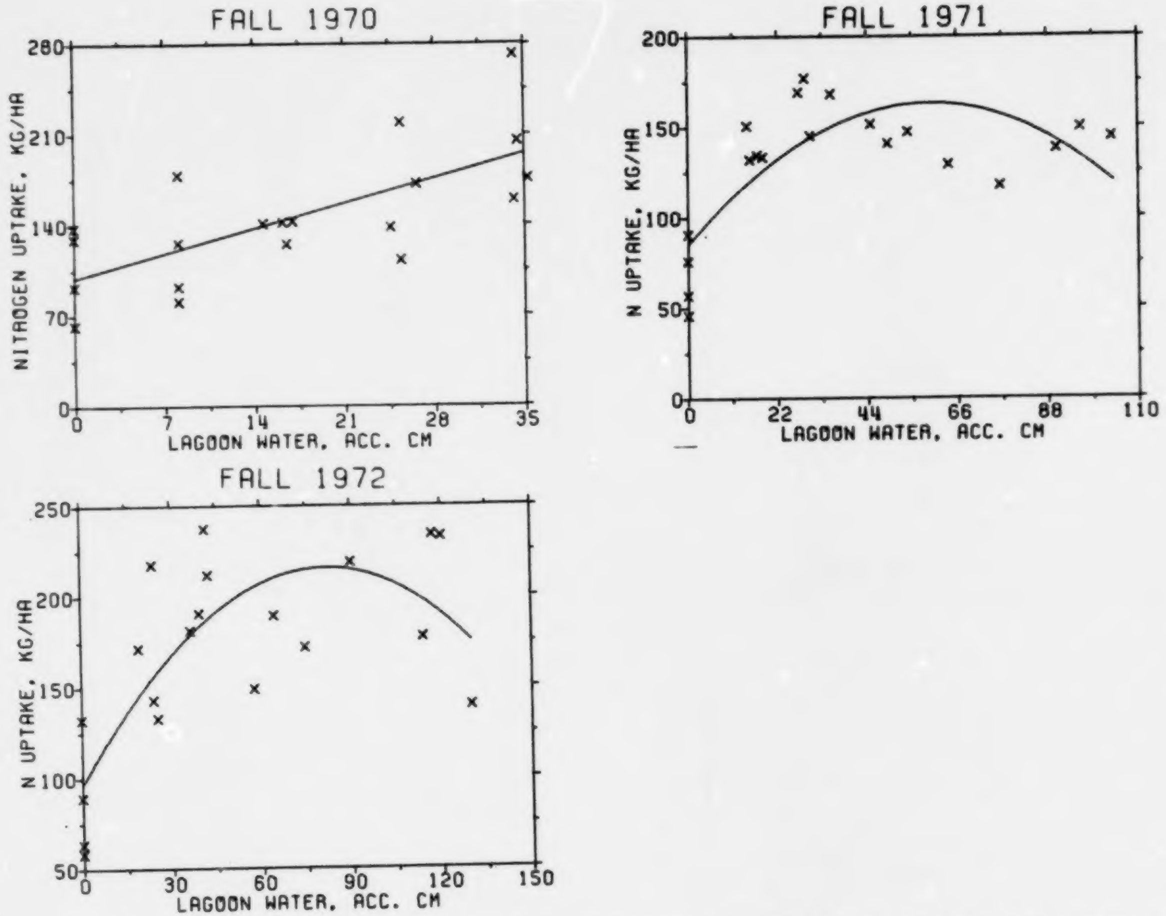

Fig. 8. Uptake of N by 1970, 1971 and 1972 corn forage as affected by accumulative applications of beef-feedlot-lagoon water.

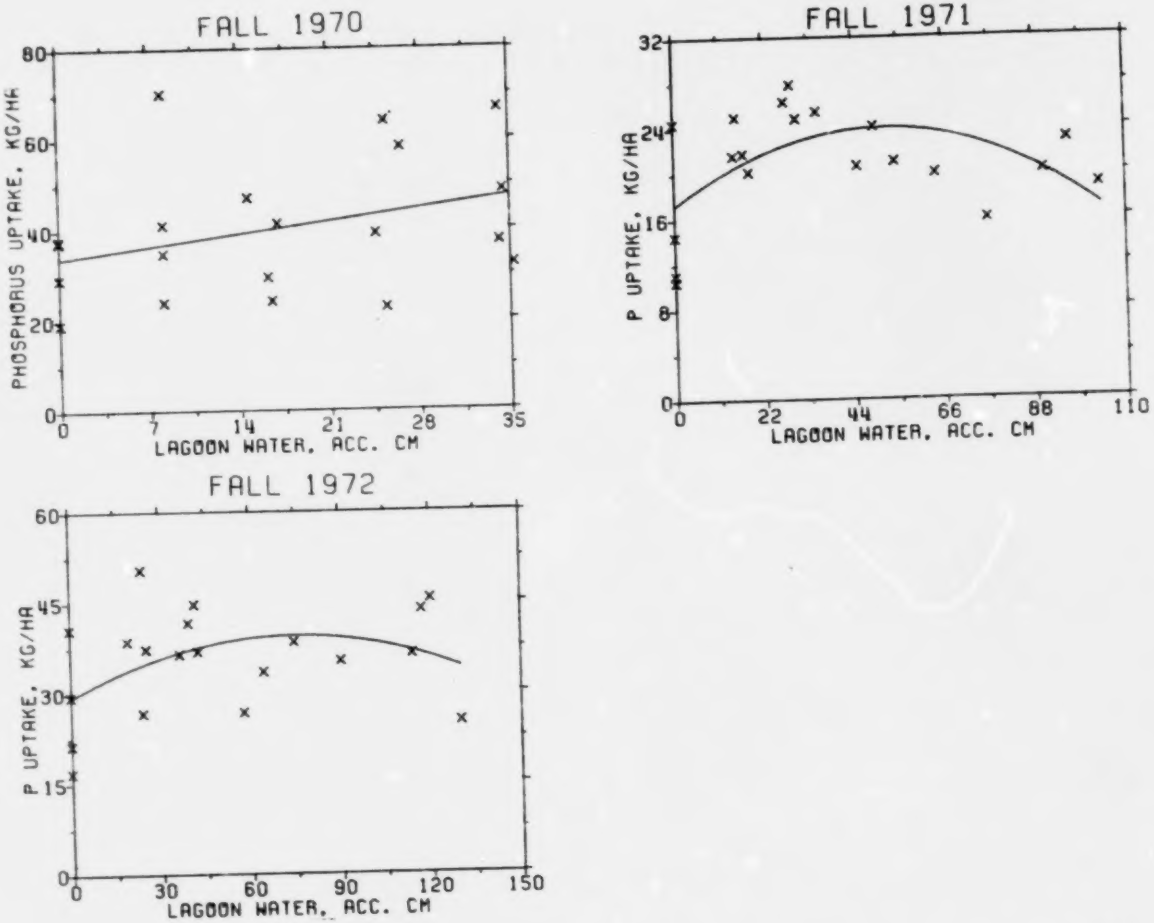

Fig. 9. Uptake of P by 1970, 1971 and 1972 corn forage as affected by accumulative applications of beef-feedlot-lagoon water.

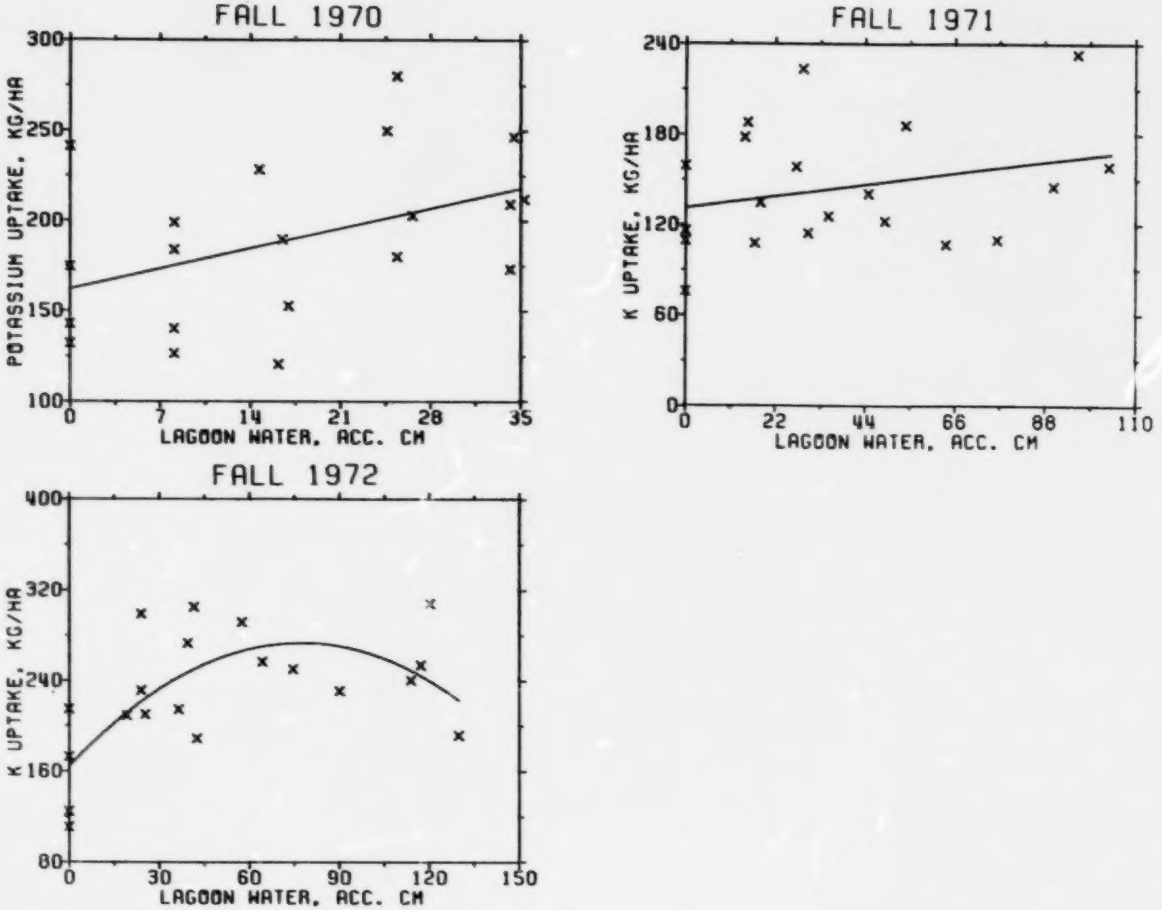

Fig. 10. Uptake of K by 1970, 1971 and 1972 corn forage as affected by accumulative applications of beef-feedlot-lagoon water.

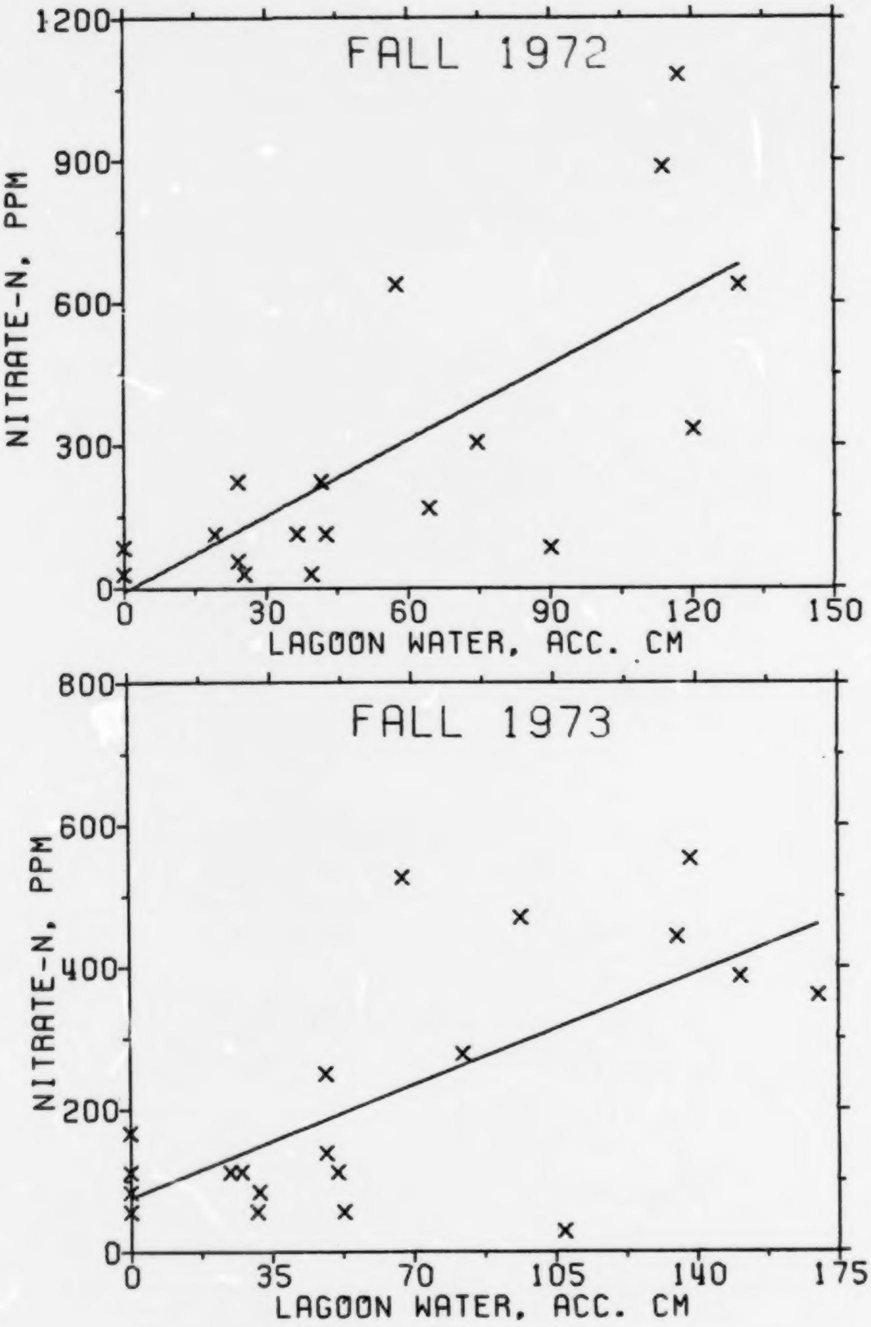

Fig. 11. Nitrate-N content (dry weight basis) of 1972 and 1973 corn forage as affected by accumulative applications of beef-feedlot-lagoon water.

IRRIGATION OF TREES AND CROPS WITH SEWAGE STABILIZATION POND EFFLUENT IN SOUTHERN MICHIGAN

by

Jeffrey C. Sutherland[a], John H. Cooley[b], Daniel G. Neary[c]
and Dean H. Urie[d]

INTRODUCTION

The "living filter" concept of sewage wastewater treatment, documented extensively at The Pennsylvania State University[1], is being tested in Michigan with systems designed both for research and practical solutions to immediate needs. Twenty to twenty-five rural projects serving populations of under 1,000 to over 10,000 are now in stages from preliminary to "in use". All of them involve initial treatment through the secondary level in facultative ponds, with or without pretreatment in anerobic or aeration ponds[2, 3].

Soil and vegetation renovation of wastewater in Michigan is a natural extension of the wide use of outdoor ponds for sewage treatment in rural communities which began around 1960. Under orders to reduce phosphorus in Great Lakes tributaries (beginning in 1968), many Michigan communities are choosing irrigation over in-plant treatment for reasons of economy.

The advantages of irrigation over other advanced treatment methods are well known: assimilation of dissolved carbon, phosphorus, nitrogen, micronutrients and filterables usually can be assured in a single treatment step. No residues need be trucked away for disposal. Increased farms yields are realized, especially in drouthy areas, and new "ground water" is occasionally a valuable water supply asset.

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Irrigation of woodlands is much less common than irrigation of farm crops. Yet woodlands, in addition to being living filter systems, have potentially great advantages: the Great Lakes region has a relatively short growing season (5-1/2 months). But year around irrigation of woodlands has been successful[1]. Public forest land often can be acquired inexpensively, or free, while costs for farm land reasonably close to service areas may be prohibitive. The expenses of annual planting and frequent mowing would be avoided in areas where there is no established agriculture to benefit from crop yields. Land too rough to farm or develop is often left in timber. Such land could be used economically for irrigation.

Isolation of residences from irrigation spray and, occasionally, from visual contact with treatment sites, has been necessary in Michigan. Woodlands can provide such isolation with a border zone tens of acres smaller than would be needed in open farm areas. Irrigation of mature stands can improve growth of understory vegetation, and thereby increase food and shelter for ground birds, rabbits and deer[4]. Woodlands on drouthy soils can absorb relatively high quantities of water without fear of tree damage, with attending increases in rate of growth being of potential benefit. In river lowlands and other wet areas, drainage improvement as part of wastewater treatment design can make or keep land suitable for tree irrigation programs. Irrigation and management of immature ornamental and shade trees to be transplanted to park, walkway and mall areas could be a practical adjunct of renewal and beautification programs.

Since all economic indicators suggest demand for wood products will grow faster than supply, increased markets for timber crops seem assured.

Studies with young tree stock were started in lower Michigan to learn how various species respond to irrigation on glacial soils common to the Lake States region, and whether there are important differences among species as to nutrient uptake. Experimental irrigation of established tree stands was begun to study growth response, water quality improvement and the effects upon existing ground water chemistry. Studies at Middleville and Belding are the first in Michigan in which sewage wastewater is being applied to trees in nursery and plantation areas. The studies in progress at Belding involve irrigation of ornamental and shade trees. Data from one season of irrigation are available from Belding, but one or more years of further study are needed for meaningful interpretations. In the Spring of 1974, irrigation of Christmas trees, pulpwood species and northern hardwood trees with pond stabilized wastewater will be started at Harbor Springs in the Little Traverse Bay area.

The availability of irrigation facilities with adjacent woodlands and areas that could be planted in trees determined the location of initial experiments. Such a facility exists at Middleville, Michigan, about 32 kilometers (20 miles) southeast of Grand Rapids. The present paper discusses the results of two years of research at Middleville.

A sketch of the treatment area is shown in Figure 1. The Middleville system (2,200 population) consists of two 4.4 ha facultative ponds and 11 ha of farm land (corn) receiving chlorinated effluent. Two experimental tree irrigation areas near the treatment site are receiving effluent from the south pond. The Middleville treatment system was designed as an irrigation agricultural program.

NUTRIENT QUALITY OF STABILIZATION POND EFFLUENT

The average nutrient concentrations irrigated are listed in Table 1. Total phosphorus (total P) is less than 4 mg/l. Total nitrogen (total N) is a little greater than 7 mg/l of which approximately 4.5 mg/l is organic nitrogen (Org-N). The qualities changed between 1972 and 1973. The nitrate nitrogen (NO_3-N): ammonia nitrogen (NH_3-N) ratio of 0.2 in 1972 rose to 2.9 in 1973 and total P fell by 40 percent in the same period. In 1972 the south pond received raw sewage directly. In 1973 the south pond received partially stabilized water from the north pond and no raw sewage. This operation change accounts for the higher NO_3-N:NH_3-N ratio. The reduction in total P is not easily explained. Retention of phosphorus in sedimented organisms is a speculative possibility.

CORN

Corn (hybrid no. 4444) is planted and harvested by a local breeder turkey farmer on 8.9 ha of the Middleville irrigation site. In making turkey feed, the corn is dried and mixed with soybeans and other grains. The corn field is mostly in Plainfield sand (Figure 1) which is naturally drouthy, infertile and easily moved by wind. Prior to the 1973 season, nitrogen rich fertilizer was added at 113 kg/ha rate. Irrigation in May through July was intermittent, but in August and September a 24-hour schedule was kept, which resulted in average applications of 68 mm/week. Corn rows are spaced at 97 cm (38 in.) and individual plants grow closely in a given row.

The results of irrigation were satisfying to the grower. Corn reached heights of 2 m and higher within the wetted perimeter; beyond the spray limits no corn exceeded 0.5 m in height. The yield on 8.9 ha (22 acres) was 221 bu/ha (88.5 bu/acre) of dry shelled corn, or roughly 500 bu/ha (200 bu/acre) of ears.

Prior to the 1974 season, nitrogen fertilizer in twice the previous amount will be applied to the fields. Liming will be done in the winter. The corn stover was plowed-in (October) in order to help build the soil.

The estimated value of the 1973 corn crop is $5,000, of which 15% to 20% is consumed in planting, harvesting, trucking and drying. Were

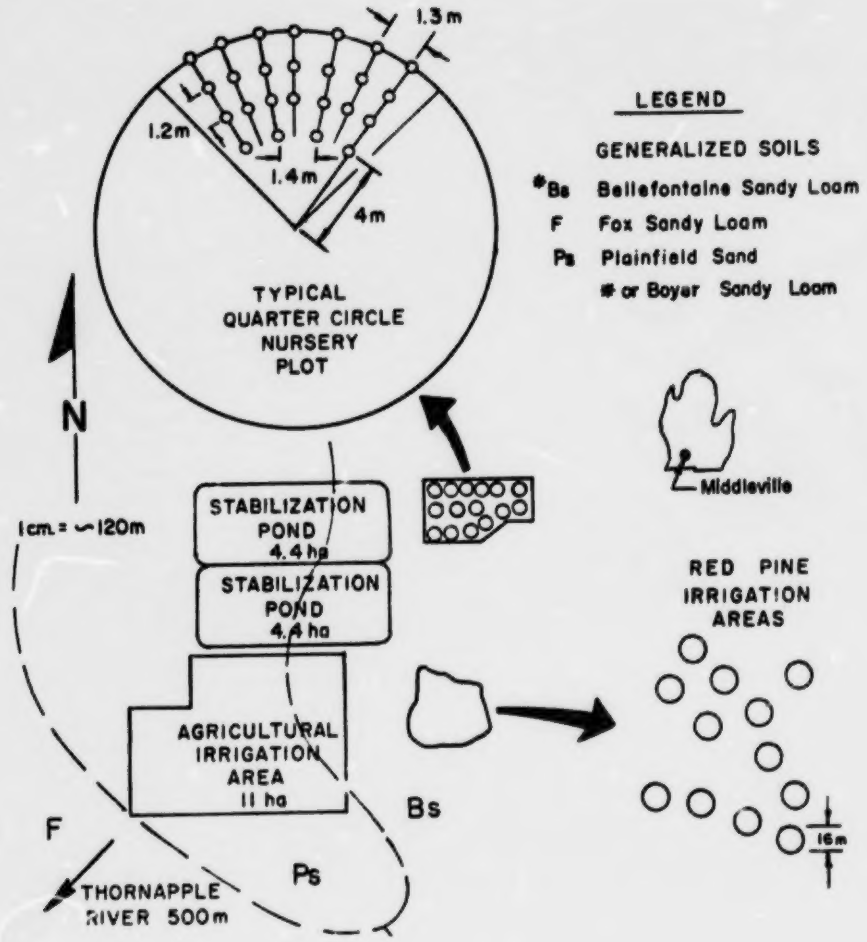
1.3m
1.2m
1.4m
4m
TYPICAL QUARTER CIRCLE NURSERY PLOT
LEGEND
GENERALIZED SOILS
*Bs Bellefontaine Sandy Loam
F Fox Sandy Loam
Ps Plainfield Sand
* or Boyer Sandy Loam
N
1cm. = ~120m
Middleville
STABILIZATION POND 4.4 ha
STABILIZATION POND 4.4 ha
RED PINE IRRIGATION AREAS
AGRICULTURAL IRRIGATION AREA 11 ha
F
Bs
Ps
THORNAPPLE RIVER 500m
16m

Figure 1. Sewage Treatment Site and Experimental Tree Study Areas, Middleville, Michigan

Table I
Irrigation and Water Quality Data by Year, 20-Year Old Red Pine Plantation, Middleville, Michigan

Parameter			1972	1973
Total Waste Water Applied at . . .		25 mm/wk	420 mm	460 mm
		50 mm/wk	820 mm	920 mm
		88 mm/wk	1350 mm	1610 mm
Average Concentration of Effluent (mg/l)		NH_3-N	2.1	0.7
		TKN	6.9	5.1
		NO_3-N	0.4	2.0
		Total N	7.3	7.1
		Total P	3.9	2.3
Average Loading Rate (kg/ha)	N	25 mm	30.7	29.8
		50 mm	59.9	65.3
		88 mm	98.5	114.3
	P	25 mm	16.4	10.6
		50 mm	32.0	21.2
		88 mm	52.6	37.0
Irrigation Season	Began		July 7	May 24
	Duration		15 weeks	*18 weeks

cept for two weeks shutdown in July for repairs due to ghtning damage.

the community to manage such an operation, the net value of the crop could pay for operation and maintenance of the ponds and irrigation system.

TWENTY YEAR OLD RED PINE PLANTATION

A stand of red pine trees (*Pinus resinosa*) established in very well drained sandy soils was irrigated during the 1972 and 1973 growing seasons. Red pine is the most common plantation species in Michigan.

Information on red pine response to wastewater irrigation is available from The Pennsylvania State University wastewater disposal studies[5]. Following ten years of irrigation with secondary level effluent, red pine receiving 2.5 cm per week exhibited increased height and diameter growth over those of unirrigated trees. Trees receiving 5.0 cm per week showed reduced growth. The growth reduction occurring at the higher irrigation rate is believed due to the combination of boron toxicity and excessive soil moisture. The red pine at Penn State grows on loam soil of the Hublersburg-Hagerstown complex. Most red pine stands in Michigan are on sandy textured soils of glacial origin having little or no clay in the sub-soil and consequent low moisture holding capacity.

Study Site

The Middleville trees grow on irregular land developed in gravelly moraine. Soil is a typic hapludalf of the Boyer (Bellefontaine) series, which is light colored, well drained sand and loamy sand overlying calcareous sand and gravel. Prior to red pine establishment, the land had been farmed and then abandoned.

The plantation has an average spacing of 2.4 m. The average basal area is 7.5 m^2/ha and the average tree height is 11.6 m. The average DBH (diameter at breast height) is 18.1 cm.

Procedures

Twelve circular areas of 7.5 m radius were selected for irrigation. All trees were marked, pruned to a height of 2.4 m and measured for DBH.

The following four treatments were randomly assigned to three areas each: (1) control - no irrigation; (2) 25 mm of effluent per week; (3) 51 mm of effluent per week; (4) 88 mm of effluent per week.

Water was applied during one 8-hour period each week by a single rotary aerial sprinkler installed at the center of each area. The amount of water applied varied predictably with distance from the sprinkler. The 4.9 m radius was selected as the point for soil water quality studies since design rates of application were most nearly met here.

Samples of sewage pond effluent were withdrawn from a tap on the irrigation line during spray periods. Soil water samples were removed from 60 cm and 120 cm depths using 51 mm diameter suction lysimeters. Vacuum was drawn on the samplers prior to irrigation and they were allowed to fill during the ensuing 7 day period.

All samples were preserved with IN HgCl (1 mg/100 ml). Analyses (total Kjeldahl nitrogen -- TKN, NH_3-N, NO_3-N and total P) were conducted by laboratories of the Institute of Water Research and Department of Forestry at Michigan State University, using standard EPA methods[6].

At the end of the growing seasons, needles from the second or third whorls from the leader were sampled from four trees in each area for nutrient analysis. Average needle length and dry weight per fascicle were determined using 100 needle groups collected from the base of the current season's shoot. The terminal bud of each branch used for needle samples was also measured. The DBH of each tree was recorded. Tree heights were determined in 1973 for both years using five representative trees per irrigation area.

Observations

The availability of percolating water at the 60 and 120 cm depths of sampling was erratic. However, sampling was usually successful at 60 cm under all three irrigation rates. The samplers placed in control plots captured so little water that only occasional analyses could be made.

The average total N and total P concentrations were calculated from mean monthly soil water data. Total monthly phosphorus values averaged 0.04 mg/l and less. Total N values were less than 1 mg/l, of which NO_3-N was 25 to 40 percent (Table 2). Higher total N concentrations in the 1973 percolate than in 1972 relate to significantly heavier rainfall, hence dilution, during the 1972 season.

The extent of nutrient renovation of wastewater was determined by weighting the concentration values by the volume of precipitation and irrigation in excess of the cumulative potential evapotranspiration[7]. Nitrogen removal through the 60 cm depth was 83 to 92 percent, and phosphorus uptake was in excess of 96 percent (Table 3). The renovation figures show increased percentages of applied nitrogen passing through the soils at heavier rates of irrigation. One probable reason for this is that heavier applications promote more rapid percolation, allowing less time for removal reactions. Levels of NH_3-N in soil water were essentially unchanged due to irrigation treatment. Nitrate-N concentrations were significantly higher under the higher rates of irrigation. Total N concentrations were also significantly higher under heavier irrigation, largely due to the higher NO_3-N. Organic forms of nitrogen accounted for approximately half of the total N. Possibly the growth of algae in the suction soil samplers converted

Table 2
Concentrations of N and P in soil water at 60 cm depth (mg/l), 1973

	Nutrient Parameter[1]				
Irrigation Rate (mm/week)	NH_3-N	TKN	NO_3-N	Total N	Total P
25	0.10 a	0.36 a	0.11 a	0.47 a	0.02 a
51	0.20 a	0.64 a	0.34 a	0.98 a	0.04 a
88	0.14 a	0.55 a	0.39 a	0.94 a	0.03 a

[1] Means not followed by the same letter are significantly different at the 2.5% level (paired student t test).

Table 3a.
Renovation of N and P in effluent (1972)

Parameters	Rate	Jul.	Aug.	Sept.	Oct.	Nov.	Total	Percent Renovation
Ground	25 mm	0	145	79	137	46	407	---
Water	51 mm	0	272	202	239	46	759	---
Recharge (mm)	88 mm	51	460	356	391	46	1304	---
Total	25 mm	0	0.6	0.2	0.5	0.2	1.5	96
Nitrogen	51 mm	0	2.6	0.8	1.0	0.2	4.6	93
(kg/ha)	88 mm	0.3	2.3	1.8	2.3	0.3	7.0	93
Total	25 mm	0	<0.1	<0.1	<0.1	<0.1	<0.4	97+
Phosphorus	51 mm	0	<0.1	<0.1	0.1	<0.1	<0.4	98+
(kg/ha)	88 mm	0.1	0.1	0.1	0.1	<0.1	<0.5	99+

Table 3b.
Renovation of N and P in effluent (1973)

Parameters	Rate	Jun.	Jul.	Aug.	Sept.	Oct.	Total	Percent Renovation
Ground	25 mm	47	0	25	152	76	300	---
Water	51 mm	74	10	152	254	152	642	---
Recharge (mm)	88 mm	112	86	343	406	255	1202	---
Total	25 mm	0.5	0	0.1	0.8	1.0	2.4	92
Nitrogen	51 mm	1.6	0.1	2.5	2.1	1.7	8.0	88
(kg/ha)	88 mm	1.6	1.3	6.0	7.5	3.0	19.4	83
Total	25 mm	<0.1	0	<0.1	0.1	<0.1	<0.4	96+
Phosphorus	51 mm	<0.1	<0.1	<0.1	0.1	0.1	<0.5	97+
(kg/ha)	88 mm	<0.1	<0.1	<0.1	0.1	0.1	<0.5	98+

NO_3-N to organic forms during the 7-day collection period.

The few successful field control samples (data not given) contained total P similar to the irrigation plot samples, indicating that no phosphorus above background levels penetrated to the subsoils.

The mean growth responses of red pine to the different irrigation rates during the 1972 and 1973 growing seasons are presented in Tables 4 and 5. Needle lengths in 1972 averaged 144.9 mm, with no significant differences due to irrigation rate. This was most likely the result of the late start in the irrigation schedule (July 15) since 90 percent of red pine growth normally occurs in June and early July[8]. In 1973 there were significant differences in needle growth which relate directly to the amount of water applied. Needle lengths increased 12, 28, and 36 percent over control areas with increasing irrigation levels, going from 121.0 mm for the unirrigated trees to 164.4 for trees receiving the greatest irrigation. The decrease in needle length in the control area trees from 1972 to 1973 is attributed to low rainfall in June, 1973 (2.69 cm), compared to that of June, 1972 (11.05 cm) which is close to the 30 year mean rainfall for June of 10 cm.

Terminal bud lengths exhibited no significant differences in 1972 which could be related to irrigation. In 1973, trees receiving 25 mm had the smallest terminal buds, but this is unrelated to the amount of irrigation since no similar reduction was evident in the higher irrigation rates. Branch terminal bud length thus proves not to be a sensitive biological indicator of red pine response to irrigation. Measurement of the terminal bud of the main stem might be a better indication of irrigation response, but it is feasible only with small trees.

The growth trends in dry weight per fascicle parallel those of needle length. No significant variations from the overall mean of 71.59 mg per fascicle were evident in 1972. The 1973 data show that the needle dry weight increased 8, 38, and 56 percent over the controls with the application of 25, 51, and 88 **mm** of effluent. The significant differences occurred between the low and intermediate application rates. Increases in both needle dry weight and length point to greater photosynthetic capability in trees irrigated at rates of 51 and 88 mm per week than those receiving less irrigation.

The anticipated result of increase in total energy fixation would be additional height and/or diameter growth. Table 6 lists the mean DBH increment from 1972 to 1973 as calculated by measuring the parameter for all trees in each plot. The average height and DBH data are included for reference. No significant increase in DBH has yet appeared. Any growth trends will show up in subsequent years as the 1973 needles assume a larger role in photosynthesis. Needles normally remain alive on trees for three years, contributing the most to photosynthesis in the second and third years.

Table 4
Mean Red Pine Growth Parameters for Varying Sewage Effluent Irrigation Rates at Middleville, Michigan, 1972

Irrigation Rate (mm/week)	Needle Length (mm)	Terminal Bud Length (mm)	Dry Weight Per Fascicle (mg)
0.0	142.6 a[1]	26.6 a	73.90 a
25	146.0 a	24.0 a	69.17 a
51	155.0 a	23.7 a	81.12 a
88	136.2 a	24.2 a	62.18 a

[1] Means not followed by the same letter are significantly different at the 5% level (Tukey's test).

Table 5
Mean Red Pine Growth Parameters for Varying Sewage Effluent Irrigation Rates at Middleville, Michigan, 1973

Irrigation Rate (mm/week)	Needle Length (mm)	Terminal Bud Length (mm)	Dry Weight Per Fascicle (mg)
0.0	121.0 a[1]	28.3 a	68.98 a
25	136.4 b	24.5 b	74.76 a
51	154.9 c	27.9 a	95.23 b
88	164.4 c	30.2 a	107.39 b

[1] Means not followed by the same letter are significantly different at the 5% level (Tukey's test).

Table 6
1973 Red Pine DBH Increments and Representative Total DBH and Height for the Sewage Effluent Irrigation Project at Middleville, Michigan

Irrigation Rate (mm/wk)	DBH Increment (mm)	DBH (cm)	Height (m)
0.0	3.99 a[1]	17.75	11.80
25	4.11 a	16.41	11.83
51	4.67 a	18.39	12.77
88	4.09 a	16.46	12.77

[1] Means not followed by the same letter are significantly different at the 5% level (Tukey's test).

The nutrient analysis of the foliage collected in 1972 indicated only one significant variation between irrigated and unirrigated trees. Only in the case of boron has a distinct irrigation rate response occurred. Sopper and Kardos[5] found boron levels of 23 and 33 micrograms/gram (ug/g) in foliage from red pine irrigated at rates of 0 and 51 mm per week, respectively. They reported no statistical significance to the observed differences. Boron levels in the foliage from Middleville specimens were 33, 28, 27 and 22 ug/g for the 88, 25, 51 and 0 mm rates, respectively. The boron content of trees receiving 88 mm of effluent per week is significantly higher than that of the unirrigated trees, and high enough that occurrence of boron toxicity conditions in the red pine is a distinct possibility. Should this condition continue and toxicity symptoms result, red pine on well drained soil may have to be eliminated from consideration as suitable for receiving treated sewage wastewater, at least at levels approaching 88 mm per week.

CUTTINGS AND SEEDLINGS

Cuttings and seedlings were planted in soils of the Boyer series developed on a moraine of gravelly till composition. Some of the test plots are positioned on slopes where erosion has removed much of the fine material leaving a gravelly sand surface soil with little capacity to retain moisture. Others are on areas where the fine material has been deposited, resulting in a mantle of fine loamy sand which has a much greater moisture retention capacity. Still others are on a temporary haul road or fill material which has been covered with roughly a foot of very stony loamy sand. A compacted substratum severely restricts percolation rate on these plots.

On the eroded slopes broadleaved weeds such as wild strawberry (Fragaria virginiana) and hawkweed (Aurantiacum spp.) were major components of existing vegetation but on the undisturbed flat areas there was a very luxuriant growth of forage grasses, mainly brome grass, and scattered low brush, mainly hoptree (Ptelea trifoliata). Clovers, fescues, and brome grass seeded on the disturbed soils had become well established before testing was begun.

Procedures

The tests included two hybrids of populus species, one an aspen type hybrid (Populus canescens X P gradidentata) and one a cottonwood type (Populus deltoides X P. nigra); two species of larch, Japanese (Larix leptolepis) and European (L. decidua); a hydrophytic conifer, northern white cedar (Thuja occidentalis); and three indigenous hardwood species, tulip poplar (Liriodendron tulipifera) which at this latitude grows naturally only on fertile, protected sites, northern red oak (Quercus borealis) which grows on more exposed slopes and ridges, and green ash (Fraxinus pennsylvanica var. lanceolata) which occurs on a wide variety of sites but reaches best development on moist bottomlands. The two populus hybrids were planted as rooted cuttings and the other species as seedlings under 45.7 cm (18 inches) tall.

The cottonwood hybrid, green ash and white cedar were planted only on the loamy sand with compact substratum; the other species were planted on all soil conditions described. The plots were rototilled thoroughly before planting.

All except the cottonwood hybrid and red oak were assigned at random to quarter circle plots of twenty-five trees each (Figure 1). The white cedar and green ash were planted in 12 quarter-circle plots; and the larches, tulip poplar and aspen hybrid were planted in 9 such plots. The cottonwood hybrid and red oak were planted in circles 8.8 m from plot centers. The cottonwood was planted in six circumferential plots, and the red oak in nine.

After the first year (1972), surviving European larch were moved to circular rows 1.5 and 2.7 m from plot centers and cuttings from the cottonwood hybrid were planted in the quarter circle originally occupied by the larch. Two cuttings were planted where each of the 25 larch had been. One was cut in February and held in cold storage for three months and the other was cut just before planting.

At intervals throughout the first growing season weeds were cut and the soil tilled. At the beginning of the second season (1973), dichlobenil (2, 6-dichlorobenzonitrile) was applied at the rate of 168 kg/ha to half of the area occupied by each species. The other half was mowed often enough to keep weeds and grass from smothering the trees.

Effluent was applied through Rainbird 24A-23^{O}FP-TNT sprinklers. A single sprinkler was used for the low rate and two on a single riser were used for the higher rate. The amount applied varied widely over any one plot due to wind drift and from year-to-year due to malfunctions in the distribution system. During the first year, the medial total amounts were approximately 420 and 685 mm for the low and high rates (46.6 and 76.2 mm/week, respectively). Irrigation did not begin until June 23, was interrupted for three weeks in July because of low water in the ponds and was terminated on September 7 to allow trees to harden off before the first frost. During the second year 330 and 889 mm were applied in total (20.6 and 55.6 mm/week, respectively) starting on May 17 and ending September 13. A two-week interruption in August due to equipment failure coincided with an extended hot, dry spell.

Observations

Variations in weather during the planting period and differences in effectiveness of weed control had a great deal of influence on survival and growth during the first growing season. Also, there was an even distribution of rainfall through most of the season which probably minimized the effects of irrigation. Nevertheless, there were differences that seemed to be related to irrigation (Table 7). Height growth of the aspen hybrid was only slightly better in plots irrigated at the low level than in unirrigated plots, but it was one third better in plots irrigated at the higher level. In contrast, the cottonwood hybrid grew nearly 25 percent better at the lower level but only slightly better at the higher level. Growth of other species was affected little, if at all, by irrigation, but survival of Japanese larch, tulip poplar, white cedar and red oak was improved. Survival of European larch was poorest in plots that received the higher level of irrigation.

Growth during the second season provides a better indication of the effects of irrigation because most tree species were well established, weed control was more uniform and there was an extended hot, dry period (Table 8).

The rooting and survival of the new cottonwood hybrid cuttings were greater under lower level irrigation (75%) than in unirrigated areas (40%) and under the higher level (68%) (Table 9). Possible development of shallow root systems in the higher level areas followed by aggravated moisture stress during the two weeks with neither rain nor irrigation could have caused the higher mortality. By the end of the season surviving cuttings in irrigated plots were more than twice as tall as those in unirrigated plots. The heavier irrigation increased height growth little more than the lighter. Survival and height growth of stored and fresh cuttings were about the same.

Table 7
Survival and Growth of Cuttings and Seedlings During the First Growing Season (1972)

Tree Species	Irrigation Level					
	None		Lower		Higher	
	Survival (%)	Height Growth (cm)	Survival (%)	Height Growth (cm)	Survival (%)	Height Growth (cm)
Cottonwood hybrid	98	50	100	62	100	52
Aspen hybrid	100	42	100	45	100	56
Green ash	95	30	96	31	100	27
European larch	76	12	80	3	65	10
Japanese larch	68	12	99	9	88	11
Tulip poplar	73	5	77	4	83	4
White cedar	79	5	87	5	91	5
Red oak	86	1	99	0	94	1

Table 8
Survival and Growth of Cuttings and Seedlings During the Second Growing Season (1973)

Tree Species	Irrigation Level					
	None		Lower		Higher	
	Survival (%)	Height Growth (cm)	Survival (%)	Height Growth (cm)	Survival (%)	Height Growth (cm)
Cottonwood hybrid 1/	100	27	100	101	100	112
Aspen hybrid	97	33	99	60	100	69
Green ash 2/	100	21	98	38	95	36
European larch	54	18	76	17	79	28
Japanese larch	94	27	92	38	89	26
Tulip poplar	52	5	75	22	80	21
White cedar 1/	86	2	94	10	86	11
Red oak	100	-1	83	-1	95	-2

1/ Difference in height growth between control and treated plots is highly significant (1% level)

2/ Difference in height growth between control and treated plots is significant (5% level)

Table 9
Survival and Growth of Cottonwood Hybrid Cuttings (1973)

Irrigation	Survival 1/ (%)	Average Height 1/ (cm)
None	40.0	33.3
Low level	74.7	70.0
High level	68.0	74.9

1/ Differences between control and treated plots are significant (5% level)

Contrary to first season results, irrigation improved survival of the European larch that was replanted at the beginning of the second season (Table 8). The greatest improvement was in plots with compact substratum. Ninety percent of the larch on unirrigated plots died compared with 75 and 32 percent with the lower and higher levels of irrigation, respectively. A similar pattern of mortality occurred in tulip poplar. Ninety-four percent of the unirrigated seedlings in plots with compact substratum died, compared with 24 percent with lower level irrigation and 20 percent with the higher level. The only unirrigated Japanese larch seedlings that died the second year had been planted on the gravelly sand. No red oak planted in unirrigated plots died even though there were some losses in irrigation plots. A few green ash died, all in irrigated plots, and there was slightly heavier mortality in white cedar but it was not related to irrigation treatments.

During the two week period with neither rain nor irrigation some mortality occurred in all irrigated plots. Most of the mortality among European larch and tulip poplar occurred in this time interval.

The irrigation effects on height growth of the two populus hybrids, tulip poplar and white cedar, were dramatic. Irrigated white cedar seedlings grew five times as much as those on unirrigated plots; irrigated cottonwood hybrids and tulip poplar grew about four times as much; and irrigated aspen hybrids grew about twice as much.

Green ash grew rapidly in all plots, but irrigation increased its growth about 80 percent. Irrigation had a similar effect on Japanese larch growing on the loamy sand, and increased growth about 35 percent in that growing on the gravelly sand. However, in the plots with compact substratum the higher level of irrigation reduced growth by 85 percent and the lower level increased growth only slightly. Irrigated European larch on loamy sand increased in height more than five times as much as in unirrigated plots but growth on gravelly sand was inferior to that in unirrigated plots. The poor growth response of larches under normally heavier irrigation in certain soils may result from greater susceptibility to drought, as speculated above for the cottonwood hybrid.

On the soil with compacted substratum, tips died back on many seedlings that received no irrigation or low level irrigation, whereas most trees grew a small amount where irrigation was heaviest. Many of the red oak died back and height growth of the others was very slow. Irrigation had no discernible effect on growth of this species.

Discussion

It is evident from the early results of this study that survival and initial growth of several tree species can be increased by irrigating with stabilization pond effluent. The long term effects of irrigation are, of course, still to be explored. There is a distinct possibility that heavy irrigation, expecially on poorly drained soils, will inhibit development of the deep root system necessary to support large trees. This could limit the size attainable in irrigated stands. There is also a possibility that insect or disease problems will be aggravated by irrigation. Diseases of root systems seem most likely to be aggravated because of higher soil moisture. These are among the factors that we hope to illuminate as we carry on additional studies including other tree species, other soil types and other kinds of distribution systems. In the near future we need to consider starting pilot tests with some of the most promising species, such as the hybrid poplars, so that we can find out more about the operational problems that may be encountered when irrigating stands on a commercial scale.

SUMMARY

Corn in drouthy, infertile soil has shown excellent response to irrigation with stabilized sewage wastewater. The net value of the crop at Middleville after farming, trucking and milling costs are subtracted could support most or all of the sewage treatment site operation and maintenance program.

The use of pond stabilized sewage wastewater for irrigation of hardwood and conifer plantings in southern Michigan has produced several distinct results after two years of treatment. Water quality monitored beneath a twenty year old red pine plantation has indicated 83-92 percent renovation of nitrogen, and 96 percent removal of phosphorus. Irrigation rates as high as 88 mm per week have resulted in an increased flow of applied nitrogen through the soil-plant system. Nitrate nitrogen levels have been significantly greater under irrigation rates of 50 and 88 mm per week than under 25 mm per week, but have remained below 1.0 mg/l. Irrigated red pine has shown increases in length and dry weight of needles by as much as 36 and 56 percent, respectively, over that of unirrigated controls. At the present time no trends in DBH increment or height growth have been observed. But with continued increases in needle length and dry weight, increases in DBH or height are anticipated. Nutrient analysis of the red pine foliage has indicated elevated levels of boron which may lead to toxicity conditions in future years. No definitive statements on the economic impact of wastewater irrigation of red pine are possible this early in the project. If red pine volume growth increases in future years, however, partial recovery of the irrigation cost should result.

The use of wastewater for the irrigation of hardwood cuttings and seedlings has produced considerable increases in survival and height growth. A cottonwood hybrid, an aspen hybrid, and green ash exhibited the most dramatic responses to irrigation. These species grew an average of 112, 69, and 36 cm, respectively, during 1973. Tulip poplar and white cedar, while producing less total growth, increased their growth by four and five times over the range of treatments. Irrigation produced moderate increases in height growth in European and Japanese larch, and little effect on red oak.

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USES OF POWER PLANT DISCHARGE WATER IN GREENHOUSE PRODUCTION[1]

by

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INTRODUCTION

AVAILABILITY OF WASTE HEAT FROM POWER PLANTS

By the term "waste" heat we mean energy which is so degraded in temperature that its economic uses are, historically, limited. Usually it has been considered practical only to discharge this energy directly to the environment. Typically, such energy appears in the large quantities of cooling water necessary to condense the steam in power generation facilities. For example, a 1000 mW fossil-fired plant with a 20° F. rise through the condenser and a 7 percent stack loss discharges slightly over 1000 cubic feet per second of warm water. Such cooling water generally is discharged in the range of 60 to 110° F., depending on the temperature of the available inlet water, the quantity circulated, plant load, and the use (if any) of supplementary cooling devices. For instance, at the TVA Browns Ferry nuclear plant, condenser water discharge is calculated to vary from a low of 70° F. in January to 110° F. in July and August, for 100 percent once-through cooling.[4] If cooling towers, either evaporative or dry, are used for part or all of the heat rejection, then the temperature of the water increases significantly. For evaporative towers, condenser water temperature would range from 75 to 125° F., depending upon the time of year and location. At Browns Ferry, predicted condenser discharge temperatures range from 110° F. in January to 124° F. in July and August, if all of the heat were to be rejected by evaporative cooling towers. For dry cooling towers, the water temperature would be 20-40° F. higher than temperature with once-through cooling.

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[1] Research sponsored jointly by the TVA's Divisions of Agricultural Development and Power Resource Planning and by the U.S. Atomic Energy Commission under contract with the Union Carbide Corporation.

[2] Division of Agricultural Development, Tennessee Valley Authority, Muscle Shoals, Alabama

[3] Oak Ridge National Laboratory, Oak Ridge, Tennessee

[4] This example is for illustration only and is not meant to imply that TVA will permit the discharge of water as hot as 110° F. directly to natural bodies of water.

The annual quantity of waste heat presently available in the U.S. is staggering--on the order of 10^{16} Btu, equivalent to 1.6 billion barrels of fuel oil. It is projected that by 1980 TVA's coal-fired and nuclear power plants will be generating waste heat at an annual rate of 10^{15} Btu.

TVA is concerned with developing all the resources in the Tennessee Valley region. Consequently, for several years we have been looking at methods of using some of this tremendous quantity of waste heat as a resource. Several projects under way are: catfish production in raceways using condenser cooling water, soil heating to extend the growing season of horticultural and field crops, and the use of condenser cooling water for environmental control in greenhouses. Waste heat will also be used in a system we are developing to biologically recycle nutrients in livestock waste.[1,2,3] Suggested uses and actual projects by other organizations are numerous.[4,5,6,7] It is doubtful that proposed systems can use more than a small fraction of the energy discharged from power plants. Nevertheless, if we could find an economic use for only 10 percent of the heat rejected from all generating stations projected to be built between now and the year 2000, the utilization would be about the same as all the electrical energy sold in 1972 (1.85×10^9 mW-hr).

PILOT GREENHOUSE

The TVA waste heat research greenhouse at Muscle Shoals, Alabama, is the result of the cooperative efforts of many individuals. Engineers at Oak Ridge National Laboratory (ORNL) developed the basic environmental control system for the greenhouse. They, in turn, provided technical assistance to TVA engineers (Division of Chemical Development) who designed the actual facility. Funds for construction of the greenhouse were provided by the Division of Power Resource Planning. The Division of Agricultural Development coordinates the project and operates the greenhouse.

The three major overall objectives of the research project are to test the capabilities of the environmental control system, to determine the effect of the resulting environment on production of horticultural crops, and to evaluate the overall economics of the system. Results of engineering and horticultural tests and economic analyses will be used to refine the system. If the resulting system proves viable, there are tentative plans to build a facility of approximately one acre at Browns Ferry nuclear power plant in north Alabama where TVA has reserved 180 acres inside the exclusion area for possible waste heat use.

Specific engineering and horticultural objectives are:

Engineering Objectives

1. To obtain controlled-environment data over the entire spectrum of yearly operating conditions,

2. To determine the response of the greenhouse to changes initiated by a relatively sophisticated control system as well as changes from external perturbations,
3. To study the effect of the presence of crops on the performance of the environmental control system (heating, cooling, and dehumidification),
4. To have an empirical check for our analytical models for greenhouse design and to improve those models, and
5. At a future date, to study alternate means of greenhouse environmental conditioning.

Horticultural Objectives

1. To obtain quantitative data on the yield of various crops,
2. To study crop performance, including the incidence and control of disease, under high relative humidity (95-100 percent) conditions,
3. To study hydroponics versus growth in rooting media, and
4. To determine the effect of root media heating on crop performance.

DESIGN

SUMMARY

The greenhouse, shown schematically in Fig. 1, is a conventional aluminum-framed glass-glazed structure. The simulation of waste heat is from an electric water heater (boiler). Cooling is by evaporation from aspen fiber pads through which air is circulated by two propeller-type fans. Air can be recirculated through an attic plenum or discharged directly outdoors. The air flow is controlled by automatically-adjusted louvers. Heating is by sensible heat transfer from warm water flowing over the aspen fiber pads to recirculated air at saturation. Dehumidification can be provided by a bank of fin-tube heaters supplied with warm water.

FUNCTIONAL REQUIREMENTS

In view of the objectives stated above, several functional requirements were identified at the start of the design and served as guides for all subsequent design work. These requirements will be discussed below.

Flexibility

Because of the research nature of the greenhouse, operational flexibility was paramount in all decisions pertaining to the heating, cooling, control and structural components and systems. This means that we tried to provide for operation where a given variable (e.g. air flow rate) departed considerably from the traditional or expected range of variation. In all cases, flexibility was deemed more important than minimal cost.

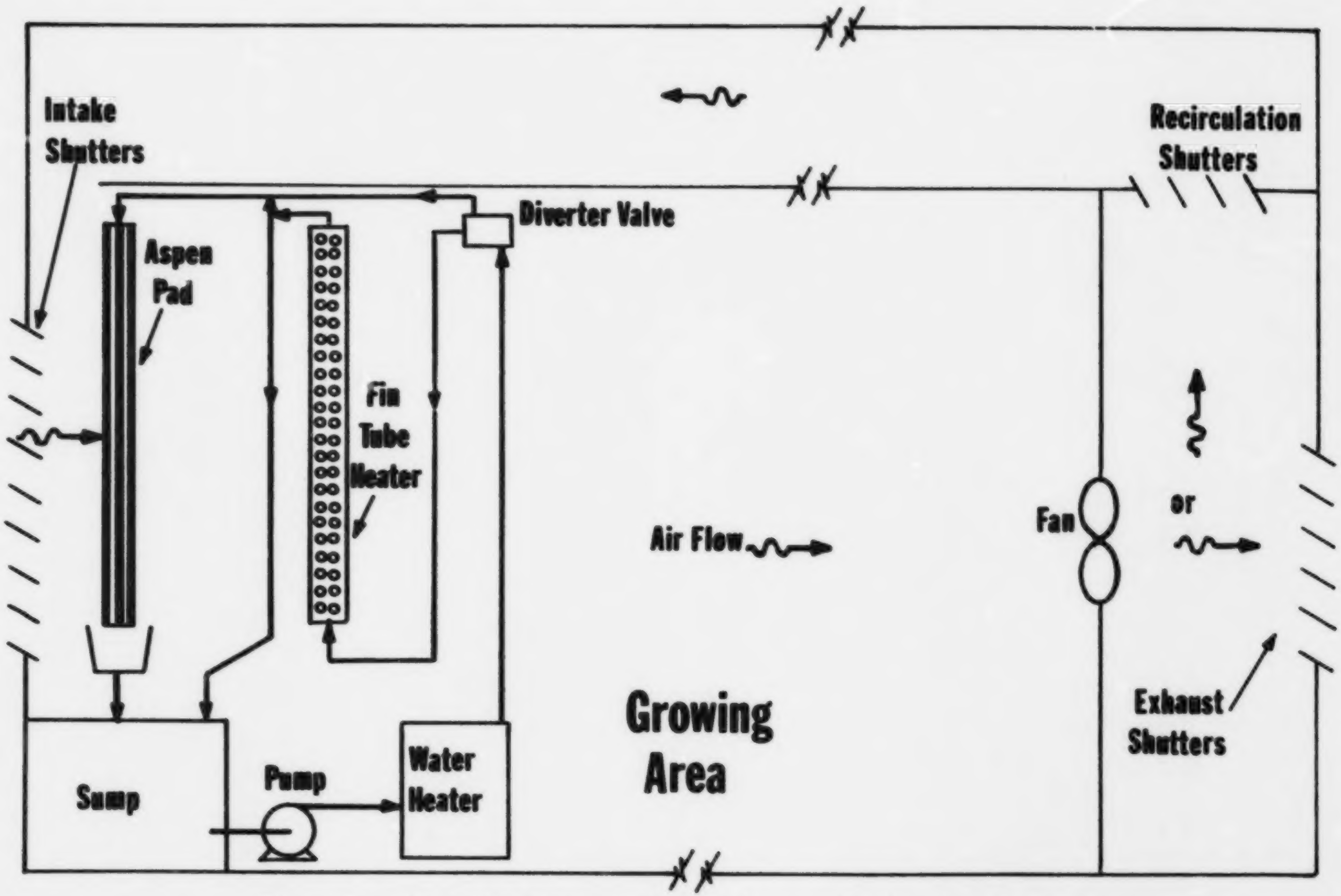
Intake Shutters
Aspen Pad
Fin Tube Heater
Diverter Valve
Recirculation Shutters
Air Flow
Fan
or
Exhaust Shutters
Growing Area
Sump
Pump
Water Heater

Figure 1. Waste Heat Research Greenhouse, Muscle Shoals, Alabama.

Major Variables

The operating variables which bear on the engineering design are summarized below, along with the design values for the range of each variable.

1. External ambient dry bulb temperature: 16-91° F.[1/]
2. External ambient relative humidity: 40-100 percent
3. Air flow rate: 11,000-36,000 cfm
4. Air exchange rate in crop region: 1-2 volumes/min.
5. Warm water flow rate: 0-100 gpm
6. Makeup water flow rate: 0-2 gpm
7. Power required for warm water source: 0-180 kW
8. Maximum relative humidity in crop region: 80-100 percent
9. Warm water inlet temperature: 70-124° F.
10. Minimum air temperature over crops: 55-60° F.
11. Maximum transmitted solar flux: 278 Btu/hr./sq. ft.

Humidity Reduction

Humidity reduction was considered a third functional requirement. This may turn out to be unnecessary, if humidity-resistant strains can be developed and disease control maintained. For example, operation close to 100 percent relative humidity has been successful at the University of Arizona Puerto Penasco Project[9]. Nevertheless, because of the research nature of our greenhouse and in the interest of flexibility, provision was made in the design for reducing relative humidity below 100 percent when operating with recirculated air or in the presence of high ambient humidity.

Data Sensing and Recording

Greenhouse temperatures, humidity, water flow rates and temperatures, external ambient conditions, fan speed, and louver bank position were selected as the major data which required detection and continuous recording.

Other Considerations

We recognize that use of warm water to heat the root zones (and, to whatever extent possible, the greenhouse air itself), constitutes a highly desirable future experiment. In addition, we desired to use one side of the greenhouse for hydroponics while the other employed rooting media. Another requirement was the provision for partial or

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[1/] The ASHRAE design guidelines[8] (for air conditioning) indicate that 54 hours per year will be below 17° F. and 150 hours will be above 94° F. at the greenhouse location.

complete recirculation of air back to the pads under ambient conditions where warm air must be conserved. These considerations were identified early so that the design would reflect any special requirements.

The detailed design proceeded after establishing the functional requirements discussed above. The sections which follow summarize the detailed design.

COOLING

Evaporation was the only type of cooling considered for the greenhouse. In this sense, the greenhouse becomes a small horizontal cooling tower when coupled to a power plant, regardless of the presence or absence of crops. The use of a bank of aspen pads approximately 23 ft. x 7 ft. x 2 in. thick follows conventional greenhouse practice. This installation is shown schematically in Fig. 1. We expect to experiment with other pad arrangements and materials in the future in an effort to improve the cooling. An important contribution to improved cooling is increased air flow through the greenhouse. This not only reduces the air ΔT through the house, due to the solar load, but also increases the heat and mass transfer coefficients in the pads themselves. The latter vary directly with the face velocity according to ORNL experiments.[10] Our fans are capable of producing two air changes per minute in the growing space. This is equivalent to a face velocity at the pads of about 250 ft./min.

HEATING AND DEHUMIDIFICATION

Due to the relatively low temperature water available, it was decided that a contact type heat exchanger would be required to supply the major portion of heat to the greenhouse. We chose to try to use the simple, inexpensive pads as contact heat-exchange surfaces for heating the greenhouse with simulated waste warm water. (Several other heating methods are available, such as sprays, underground pipes, compact fin-tube radiators, and even heat pumps. These alternate methods will not be discussed in this paper.)

Two precedents existed for the direct-contact heating--the experience at Puerto Penasco by the University of Arizona researchers and our own experience at ORNL with a small test greenhouse.

One feature of the direct-contact mode of heating is that the air quickly reaches saturation and remains near that condition as it is recirculated back through the pads and the greenhouse. To permit dehumidification, we installed a bank of fin-tube heaters about 5 ft. downstream of the pads (Fig. 1). This heater bank is spread out to cover the entire flow area. Each tube is about 20 ft. long, headered at each end to form two circuits of four passes each to provide even temperature distribution. The fin-tube bank can be operated with warm water either in series or in parallel with the pads, as shown in Fig. 1.

It is designed to lower the relative humidity from 100 percent to 80 percent by providing 70 kW. One-inch copper tubing is used with aluminum fins. Air pressure drop across the surface is negligible.

Water at waste heat temperatures for the pads and fin-tube heater is provided by a standard commercial 180 kW hot water heater.

Heating of the plant root zones is accomplished by diverting warm water from the system just downstream of the hot water heater. This water flows through coils of 3/4 inch PVC pipe buried 8 in. below the surface of two rooting media (soil and pine bark/vermiculite mix). The water is discharged to the pump suction basin.

CONTROL AND INSTRUMENTATION SYSTEM

The control system is designed to maintain a preset air temperature and a preset relative humidity with a given fixed supply water temperature.

Air temperature is controlled by changing fan speed and by modulating the amount of air that is recirculated through the house. The possible air flow modes are: once-through, and 25 percent, 50 percent, 75 percent and 100 percent recirculation. Control is achieved by individually opening or closing each of four banks of louvers on the inlet, outlet, and recirculation flow paths. Five thermistors in the greenhouse supply temperature signals; control can be from any one or combination of the five.

Relative humidity control is effected by diverting warm water through the fin-tube heater. A diverter valve (Fig. 1) modulates the flow in response to a signal from two gold-grid humidity detectors in the growing space.

Instrumentation for the greenhouse consists of detectors for flow, temperature, and humidity signals plus appropriate readout and, in some cases, recording equipment.

The warm water supply temperature is preset on the water heater. Water temperature is measured by thermocouples at six locations: upstream of the water heater, downstream of the mixing valve, outlet of the fin-tube heater, pad inlet and outlet, and makeup water inlet. These values are recorded on a multi-channel strip recorder. Ambient air temperature and five internal greenhouse temperatures are measured by thermistors and similarly recorded. Five portable recording hygrometers have been used initially for both wet and dry bulb data, but they will not be retained for long-term operation.

Relative humidity is measured by two separate gold-grid detectors mounted in aspirated cabinets at the entrance and exit of the growing space. These signals are fed to a controller which has a provision for recording, but they are not recorded at present. A visual indication is provided on the control panel, however.

Fan speed and louver position have visual indicators on the control panel and are also recorded on a strip-chart device. The TVA weather station at Muscle Shoals can provide hourly summaries of wind speed and direction, dry bulb and dew point, barometric pressure, and, most significantly, solar and total radiant flux. We expect to utilize much of these data in lieu of duplicating the same measurements ourselves.

INITIAL ENGINEERING TESTS AND RESULTS

One design error had to be rectified: the attic barrier, originally 4-mil polyethylene, was replaced with corrugated fiber glass-reinforced acrylic panels. Over a period of about 8 months, the polyethylene had become severely embrittled and weakened by the combination of solar radiation and heat in the attic. Also, an exhaust fan may be installed for the attic, to operate when the main fans are in the once-through mode (summer cooling).

TESTS

In general, our engineering tests sought to measure:

1. Air flow rate, both fast and slow speed, with once-through flow and with partial and full recirculation. These data were taken with a hot-wire anemometer using a 12-point grid set up to represent equal flow areas per point.
2. Fan performance, such as speed, head, flow and power requirement,
3. Pressure drops, especially for the pads, fin-tube heater, and attic,
4. Air temperature and humidity at various locations and under various operating conditions,
5. Control system performance, such as the maintenance of preset temperature or humidity,
6. Water temperature as controlled by the water heater, and
7. Pad and fin-tube performance, as measured by water temperatures and air enthalpies.

RESULTS

Our engineering tests are not complete as yet, but a summary of qualitative results is presented below. (Items 1, 2, 3 and 8 apply to an empty greenhouse. The others apply to a house with a mature cucumber crop.)

1. Air flow decreases as the fraction recirculated increases, with 80 percent of the once-through flow obtained under full recirculation. This applies to either fan speed.
2. There is considerable variation in air velocity with location in the plane perpendicular to flow. Flow decreases as elevation increases, with a change as much as 50 percent in some locations.
3. The water heater, with its 10-step controller, maintains water temperature within ±2° F. of the setpoint.
4. The greenhouse air temperature ranged between 65 and 68° F. for a clear night where the ambient reached a 19° F. while operating with water at 74° F. The fin-tube heater supplied about 20-30

percent of the heat under these conditions.

5. Data taken over seven days in early December, with ambients ranging from 19 to 35° F. indicated air temperatures 7 to 11° F. lower than water inlet temperatures to the pad. (The fin-tube heater was supplying about 20-30 percent of the total heat during this period.)
6. The coldest night to date, 13° F., produced a 63° F. house temperature with 74° F. water. The fin-tube heater supplied about 20-30 percent of the heat.
7. We have calculated that 95-100 kW of heat was used when the ambient temperature reached 19° F. This is in good agreement with the design value of 111 kW at 16° C.
8. Greenhouse cooling was checked at two extremes of water temperature, 66° F. and 119° F. In the former case, the water was recirculated and the pad inlet and outlet temperatures were the same as the ambient wet-bulb 66° F. The switch to high-temperature water brought the pad outlet up to 73° F. In both cases, the ambient air was cooled, by 6° F. with 66° F. supply water and by 4° F. with the 119° F. water. Further cooling data must await spring and summer operation.
9. The heating system is highly buffered against changes in outside temperature as evidenced in Fig. 2. A change of 31° F. outside resulted in only a 5° F. change inside.

INITIAL HORTICULTURAL TESTS AND RESULTS

We are testing tomatoes, cucumbers and lettuce in the greenhouse. These three crops were chosen because they occupy about 93 percent of the greenhouse area devoted to vegetable production in the United States. In one experiment, rooting media are being tested: heated and unheated soil (Ochlockonee fine sandy loam), heated and unheated pine bark/vermiculite mix, and wheat straw bales (cucumbers only). The pine bark/vermiculite mix is a variation of the peat moss/vermiculite mix commonly used for greenhouse tomatoes. The straw bale culture is a technique commonly used in Europe and England for greenhouse cucumber production. All media treatments received fertilizer according to the schedule in Table 1.

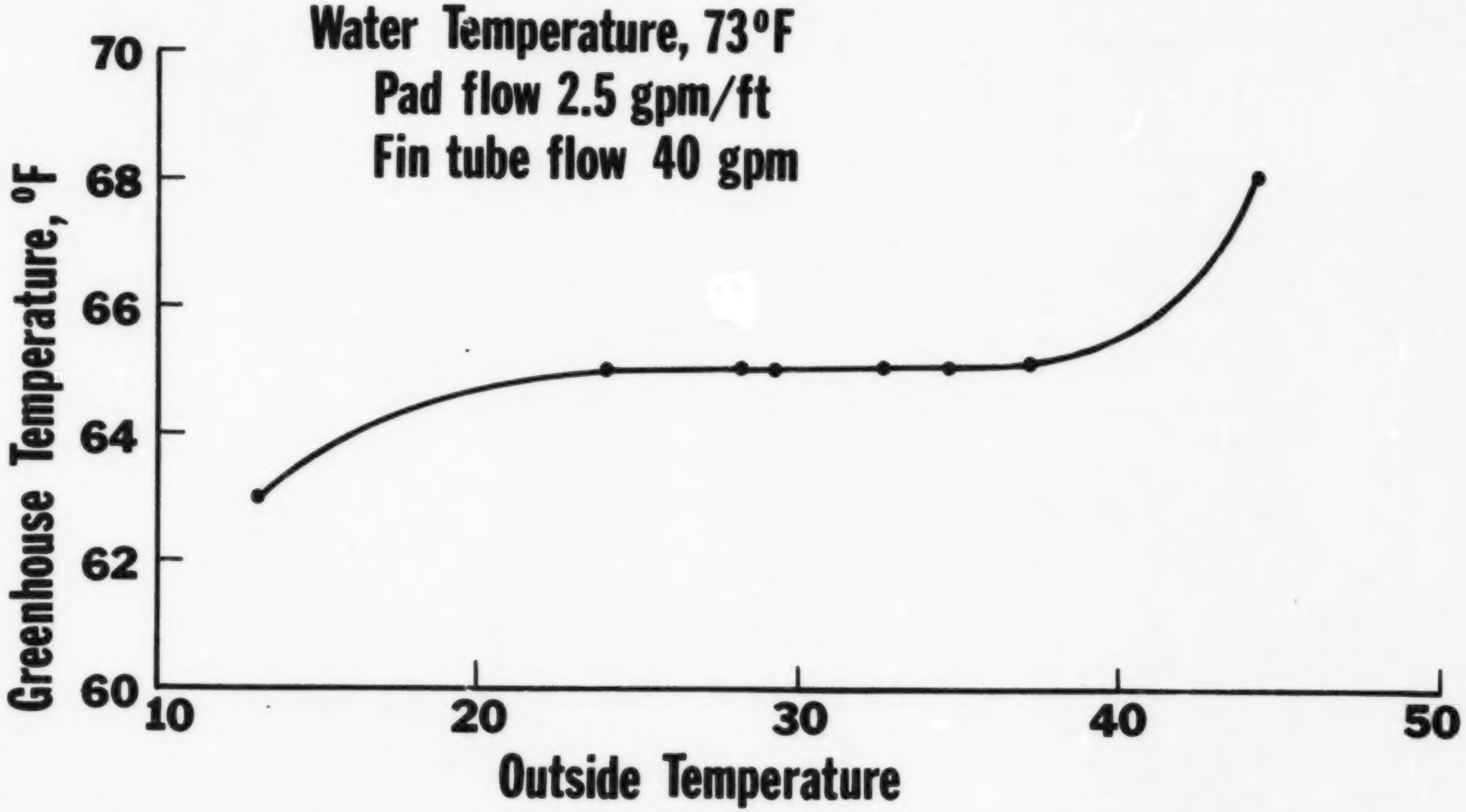

Figure 2. Response of greenhouse temperature to outside temperature changes.

Table 1. Fertilizer Schedule[1] for Rooting Media

Week	$(NH_4)_2HPO_4$	KH_2PO_4	KNO_3	$Ca(NO_3)_2$	NH_4NO_3
	grams/100 liter				
1	134	134	0	0	0
2-3	67	67	0	0	0
4-10	67	67	134	268	134
11-12	134	0	268	268	134
13-17	134	0	268	268	268

[1] 400 ml of solution/plant applied twice per week.

In a separate experiment, two hydroponic systems are being compared. One system consists of gravel-filled troughs which are periodically pumped full of nutrient solution and then allowed to drain. They are filled four times per day during clear weather and fewer times during cloudy periods. The other system consists of troughs of coarse sand to which nutrient solution is surface applied daily. Both systems use the nutrient solution shown in Table 2.

Table 2. Nutrient Solution Used in the Hydroponic Systems

Element	Concentration ppm
N	135 (increased to 200 after first fruit set)
P	62
K	156
Ca	165 (increased to 230 after first fruit set)
Mg	49
S	64
Mn	0.62
Cu	0.05
Mo	0.03
Zn	0.09
Fe	5.00
Cl	0.86
B	0.44

Femfrance cucumbers were transplanted into the greenhouse on October 10 at a population density of one plant per 8 ft.^{2} of greenhouse area. Femfrance is a long, seedless cucumber developed especially for greenhouse production. Yields for the entire harvest period are shown in Table 3.

There was no significant effect of media heating on yield. This is because soil and mix temperatures have been at an acceptable level to date. For example, on December 3 with 84° F. water, the mix was 74° F. unheated and 82° F. heated. Respective values for soil were 74° F. and 80° F.

There was no significant effect of media on total or grade no. 2 yield. However, yields of grade no. 1 cucumbers in bale culture were less than yields in soil or artificial mix. Also, the unheated soil was superior to artificial mix in number of grade no. 1 cucumbers produced.

Hydroponic yields cannot be statistically compared to the other yields. Significantly fewer grade no. 2 cucumbers were produced in the sand culture as compared to the gravel culture. Type of hydroponic system had no effect in the other two yield categories.

Grand Rapids leaf lettuce and Bibb lettuce were transplanted into the greenhouse on November 15. The hydroponic systems and the heated and unheated soil and mix used were identical to those used for cucumber production. In late January, cucumbers were replanted and the lettuce replaced with tomatoes.

During November, the greenhouse was operated without humidity control for 17 days; i.e., the fin-tube heater was inoperative. During this period of high humidity (95 to 100 percent relative humidity), there was an outbreak of powdery mildew (Erysiphe cichoracearum). A reduction in humidity to about 85 percent by activating the fin-tube heater and a change from zineb fungicide to benlate checked the fungal outbreak. It is probable that simply the use of benlate would have had the same result.

Since Femfrance cucumbers are different in appearance and taste from cucumbers available in stores in the Muscle Shoals area, a consumer acceptance test was conducted at a local supermarket. Results are given in Table 4. The Femfrance cucumbers were well accepted and sold for about twice the price of the field variety.

Table 3. Cucumber Yield, Nov. 8-Jan. 30.

	Soil		Pine Bark Vermiculite Mix		Straw	Hydroponic	
	Unheated	Heated	Unheated	Heated	Bales	gravel	sand
(1) Total yield: lbs./plant	19.4	18.2	18.4	19.1	14.1	21.2	22.3
Cucumbers/plant	16.1	14.9	14.0	14.7	11.7	18.0	19.5
(2) Grade no. 1 yield:							
lbs./plant	15.6a[1]	15.5a	13.6a	13.9a	8.6b	16.8	20.1
Cucumbers/plant	12.5a	12.3ab	9.9c	10.3bc	6.6d	13.7	17.3
Average weight, lbs.	1.25	1.26	1.37	1.35	1.30	1.23	1.16
(3) Grade no. 2 yield:							
lbs./plant	3.4	2.6	3.6	4.7	4.2	3.9a	2.2b
Cucumbers/plant	3.2	2.4	2.9	3.9	3.7	3.7a	2.2b
Average weight, lts.	1.10	1.10	1.26	1.21	1.13	1.0	1.0

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[1] Values followed by the same letter are not significantly different at the 5 percent level of probability. Hydroponic yields cannot be statistically compared with yields from other treatments.

Table 4. Market Test, Nov. 30-Jan. 18

	Price		Number
	Range	Mean	Sold
	- - -	$ - - -	
Femfrance	.25-.49	.38	406
Field Variety	.15-.29	.20	1013

ECONOMIC IMPLICATIONS

Results of an initial economic evaluation stress the need for refinements in greenhouse design to make it show greater economic potential. One example would be to recirculate the air through an adjacent house and thus, eliminate the fiber glass ceiling presently used to form the recirculation attic. Also, if power plants go to closed-loop cooling, the higher temperature water available will lower capital and operating cost. For example, lower water flow rates or smaller heat exchangers would be required. Also, cost of disease control would be reduced if lower humidity could be maintained with the warmer water. Of course, many factors which would improve production and sales in a conventional greenhouse would also be important in improving the economic potential of waste heat greenhouses. These factors would include improved crop varieties, better and cheaper disease and insect control, improved labor efficiency, and the increasing demand for off-season, greenhouse grown products.

IN CONCLUSION

We are pleased with the engineering system because it has performed as designed to date. However, we do plan modifications to the present house to test variation on the central theme of heating and cooling with a single contacting device (pads). Similarly, horticultural production has been satisfactory to date but we will continue screening crops, varieties, and rooting media to increase production.

An important future question which we intend to examine is that of the interface between a large greenhouse installation and a power plant. Of interest are such things as variation of water temperature with load, plant shutdown, water quality and activity monitoring, greenhouse revenue and rate structure, legal liability to commercial lessees, etc. In addition, many questions remain regarding crop marketing and operational economics.

We believe that this pilot facility, although still in the early stages of operation, will continue to provide us with good engineering guidelines for the design of a larger demonstration facility to be located at an operating power plant, and we look forward to initiating conceptual design work on such an installation.

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PART 5 NONTECHNICAL RESTRAINTS

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LEGAL CONSTRAINTS ON THE USE OF WASTEWATER FOR FOOD AND FIBER

by

William R. Walker and William E. Cox*

INTRODUCTION

The world demand for increased production of food and fiber places an ever increasing strain on the available water supply. The problem is accentuated by the current energy crisis, which is not likely to be of short term duration. Prior estimates of water demands may well be grossly underestimated when one contemplates the consumptive use of water in energy production (e.g., coal-gasification) and for irrigated agriculture. Effective management of our wastewater resources moves from something which is highly desirable to something which is absolutely essential.

The recycling of municipal sludges and effluents on land has been used more extensively in other parts of the world than it has in the continental United States; therefore, this alternative for waste disposal has not had an in-depth evaluation in the United States relative to other methods. Increased interest in recent years has stimulated additional research into the health aspects of this method of disposal (e.g., survival of viruses) and the direct effect on food and fiber production. No assessment of this waste management technique can be complete, however, without some discussion of the institutional processes by or through which this waste management scheme functions. Although these institutional considerations include such diverse matters as the form and powers of water organizations, financial arrangements, public attitudes, and political traditions, this paper will concentrate on legal aspects as manifest in legislative enactments and the common law. It will first examine direct government control over this disposal technique by the federal and state levels of government. Second, since relatively large areas of land are an indispensable component in this method of wastewater treatment, governmental regulation of land use must be examined along with the corollary common law rights of other property owners in the same area. Third, both statutory and common law rights related to water must be fully analyzed in terms of this method of disposal since land application of wastewater alters the flow regimens of both surface and ground water.

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DIRECT GOVERNMENT CONTROLS

FEDERAL

The federal government has historically left the jurisdiction of water pollution control in the province of state and local government, viewing it essentially as a public health matter to be dealt with under the police powers. The first comprehensive federal control effort was the passage of the Water Pollution Control Act[1] in 1948. This legislation relegated the federal government to a "back-up" position with primary responsibility for pollution control to remain in the hands of the states. The Federal Water Pollution Control Act of 1956[2] contained the first authorization of federal grants on a large scale to assist states and municipalities in planning and building facilities for treatment of wastewaters. The Act contained prohibitions and omissions that were not encouraging to the land treatment method. For example, the cost of land for sewage treatment, including use of land as an integral part of wastewater processing, was not eligible for grant assistance, and no provisions were made for grants in connection with recycling and reclamation of wastewater.

On October 18, 1972, the Federal Water Pollution Control Act Amendments of 1972[3] became law. This Act is without question the most comprehensive and complex legislation that has ever been enacted to clean up the nation's waters. It establishes a national policy that a major effort be made to develop technology necessary to eliminate discharge of pollutants into the navigable waters, waters of the contiguous zone, and the ocean, and several specific provisions of the legislation give impetus to consideration of land disposal as a serious alternative for alleviating the water pollution problem.

One of the basic encouragements is the requirement that publicly-owned treatment plants achieve secondary treatment results by 1977[4], including removal of all floatable solids and 85 percent of suspended solids. In addition to increased costs in general, this requirement will result in greater quantities of sludge to be disposed of, a task made more difficult in some cases by restrictions on ocean disposal of sludge contained in the legislation. Thus an incentive for land application of wastewater is produced since this technique has the capability of meeting the secondary treatment requirement and handling a part of the sludge disposal problem as well.

Land treatment is also facilitated by provisions of the 1972 Amendments which permit the use of federal grants for acquisition of land that will be an integral part of the treatment process or is used for ultimate disposal of residues resulting from treatment operations[5]. Grants cannot be used to acquire land upon which a conventional treatment plant is located.

Provisions of the Act and regulations adopted for its implementation show a definite intent that land treatment be considered as an alternative form of waste treatment. For example, the legislative provision requiring the Administrator of the Environmental Protection Agency (EPA) to encourage the development of revenue producing waste treatment facilities makes specific reference to recycling potential sewage pollutants through the production of agriculture, silviculture, or aquaculture products[6]. Another relevant provision is included in the proposed regulations containing cost effectiveness analysis guidelines. This provision requires the consideration of all feasible alternatives, specifically including systems using land or subsurface disposal techniques[7].

Although this most recent manifestation of federal water quality policy suggests and even encourages the use of land disposal where it is most cost effective, primary responsibility still resides with the states subject to the Administrator's approval of the state's permit program[8].

STATE

Land application of wastewater is generally subject to state control as are other methods of waste disposal. Acceptance of this treatment concept varies among the states. A 1972 study by Temple University indicates that 14 states had a favorable orientation toward land treatment and 11 were negative in their outlook, with the remaining 25 either neutral or not subject to classification on the basis of available information[9].

Control over land treatment facilities has largely been provided within the discretion of the state regulatory agencies, with few formal regulations having been adopted. A survey reported in a 1973 EPA publication conducted by the American Public Works Association with state health and water pollution control agencies indicates that most state agencies have no set policies on this phase of wastewater handling or attendant environmental impacts, do not impose specific conditions on installations, seldom inspect existing systems, and seldom require monitoring procedures and the filing of official reports on operation. Only five states indicated that official regulations governing irrigation with wastewater were in effect: Arkansas, Arizona, Colorado, New Mexico, and Texas. Only four states, Arizona, Arkansas, New Mexico, and Texas, indicated that they have rules governing the type of crops approved for wastewater irrigated lands. Permitted crops range from "forage only" to "all types." Texas regulations demonstrate concern over consumption of raw crops from waste-contact areas, with a prohibition in effect against wastewater irrigation of those crops to be consumed in the raw state. The few states which invoke crop restrictions also specify the quality of effluent to be applied to the land[10].

Other states in addition to those listed as having regulations governing wastewater irrigation have controls with regard to land treatment in general. For example, California[11] and Idaho[12] have regulations of this type. These controls would appear to apply to any proposal for wastewater irrigation.

LAND USE CONTROLS

Policy concerning land use control is currently undergoing considerable re-evaluation as indicated by efforts to enact federal land use planning legislation and the establishment of land use control mechanisms at the state level. Provisions of these new laws and regulatory procedures will likely impose direct controls over land application of wastewater, but until these programs are implemented, the traditional controls imposed by local zoning ordinances and private control measures will provide the most significant constraints.

The applicability of zoning ordinances to land treatment facilities is a complicated matter because of dependence on such factors as the provisions of enabling legislation and the terms of the particular zoning ordinance involved in a given situation. Two different situations may arise in which the applicability of zoning would be significant. The first concerns the extent to which a governing body is subject to its own zoning restrictions, and the second involves application of zoning restrictions of a second governmental entity where facilities are to be constructed on land lying outside the jurisdiction of the owner.

With regard to the question of whether a governmental body is subject to its own zoning restrictions, the answer is frequently provided by provisions in zoning ordinances which exempt governmental uses from their provisions. For example, The Supreme Court of Pennsylvania in a 1952 case[13] upheld the right of a municipality to construct a sewage treatment plant in a residential district since the zoning ordinance specifically permitted "municipal use." Where no specific exception is made for governmental use, the decision of whether the restrictions apply is sometimes based on a distinction between proprietary and governmental functions. Proprietary functions are generally held to be subject to zoning restrictions while governmental functions are not[14]. In general, government functions are those imposed upon a municipal corporation by the state as a part of the sovereignty of the state to be exercised by the municipality for the benefit of the general public, both within and without the corporate limits. On the other hand, proprietary functions are those exercised with respect to a municipality's private rights as a corporate body for the advantage of the inhabitants of the city and of the city itself[15].

Of course, the key question which arises is whether operation of sewage treatment facilities is proprietary or governmental in nature, a determination which may have varying outcomes. In a 1935 New York case[16], a municipality was enjoined from erecting a garbage disposal plant in a restricted district on the grounds that it was performing a proprietary act and therefore bound equally with all other persons by the terms of its own ordinance which prohibited disposal plants. An opposing viewpoint is expressed in a 1969 Vermont case[17] which holds the construction of a sewage disposal plant to be a governmental function and therefore exempt from local zoning ordinances.

Variation in outcome also characterizes attempts by one governmental body to apply zoning restrictions to sewage treatment facilities to be operated within its jurisdiction by a second political entity. An example of a case of this type where zoning restrictions were upheld is given by a 1962 decision of the Supreme Court of Missouri[18]. An injunction was awarded to a county for prohibition of the construction of a sewage disposal plant by a city in a residential district of the county. The opposite result was reached in a 1962 Arizona decision[19] in which the state supreme court held that the operation of a municipal sewage disposal plant was exempt from zoning regulations of another municipality in which the property involved was located. After recognizing a division of authority as to whether sewage disposal is a proprietary or governmental function, the court took note of the fact that the weight of recent authority appears to support the theory of a governmental function. This trend indicates that municipalities are not likely to be inhibited by internal or external zoning in the location of land disposal facilities in an increasing number of localities.

In addition to formalized land use controls, land treatment facilities may also be subject to private control measures where such operations have a detrimental impact on surrounding property. Of interest here is the system of civil law which defines private rights and provides a mechanism for accountability where the activities of one party injure or infringe upon the rights of others. This law is embodied in the accumulated decisions of the courts and is enforced by means of litigation, which may take the form of an action for damages or suit for injunction.

The concept of private nuisance is likely to be a central element in any legal conflict arising out of situations where land treatment facilities create conditions which unreasonably interfere with the use or enjoyment of other property. Land treatment facilities are not likely to be declared a nuisance simply because of the displeasure of an adjacent landowner; substantial interference with a legally protected interest is required. In determining whether a given facility does constitute a nuisance, the court will weigh the gravity of the harm produced against the social utility of the activity in question. Since the utility of waste treatment to the general public cannot be questioned, suits based on nothing more than a general objection to the establishment of such facilities almost invariably have been unsuccessful[20]. Another effect of the relationship of such operations to the public interest is the refusal by the courts in most cases to grant injunctions that would prohibit construction or force cessation of operations[21]. Although cases exist where sewage disposal plants have been enjoined[22], the more common remedy imposed by the courts is an award of compensable damages.

A successful suit for damages must be based on a showing of injury in excess of slight discomfort or annoyance, but it is not necessary that the property in question be made totally unusable or that an imminent danger to health exist in order for a legal course of action to arise. The concept of nuisance encompasses activities that create conditions offensive to persons or ordinary sensibilities, including unpleasant odors, loud noises, and disease-causing insects. The courts have generally recognized the right to an action for damages where these conditions are caused by the operation of sewage disposal facilities[23]. Damages which can be collected include compensation for depreciation of property values and an award for personal discomfort[24].

If nuisance conditions are created by a waste treatment facility, the right to recover damages is not likely to be nullified by the claim that the facility has been properly designed and operated. A 1942 Iowa court decision[25] addresses this issue with regard to a conventional sewage treatment plant. The owner of the plant maintained that liability should not be imposed because the plant involved was of the most modern type, had been designed by a competent engineer, and had been given approval by a state agency. The court held that these factors did not relieve liability arising from the detrimental impact on other property. To the extent that proper design and operation of facilities actually prevent adverse effects, the likelihood of legal action is reduced, but compliance with such procedures is no shield from accountability for injury produced.

In addition to the landowner whose use exists at the time of establishment of land treatment facilities, the owner who initiates a use of near-by property subsequent to installation and operation of treatment facilities also is likely to possess the right to maintain a legal action for compensable damages in connection with property injury. "Coming to the nuisance" is generally no bar to an action for recovery. This principle has been applied to a variety of industrial or commercial activities which were originally established in undeveloped regions but were later encompassed by expanding urbanization[26]. It has been indicated that public facilities are also subject to such action. For example, the court in a 1951 Florida decision[27] which denied a request for an injunction noted that an adequate remedy for damages existed for actual injury to property produced by a garbage disposal plant which had been encompassed by urban growth.

CONSTRAINTS IMPOSED BY WATER RIGHTS

The third basic category of potential legal constraints on land application of wastewater consists of adverse effects on private water rights that may result from alteration of water quality and natural flow patterns. Although land treatment is intended to reduce overall water pollution, any degradation of ground water quality may subject the operator to legal action by ground water users. In addition, ground water recharge may produce damage to others where the water table is artificially raised. With regard to surface waters, the principal adverse effect of land application is the reduction in streamflow which may occur below the previous point of discharge and result in injury to water users located there. Thus it is necessary to consider several aspects of private water rights which may constrain use of land treatment.

GROUND WATER

Perhaps the most obvious legal constraint on land application of wastewater imposed by ground water law concerns protection of quality. Although it is generally believed that the majority of pollutants in wastewater will not survive oxidation in the soil, it is likely that certain residuals and trace contaminants will find their way to the ground water. In some cases, the end result of the oxidation process itself may be a form of contaminant.

The right of the landowner to uncontaminated ground water has not always been recognized. The courts in some cases have been reluctant to impose liability where ground water pollution has resulted from lawful uses of land on the basis that such injury could not have been anticipated[28]. In other cases, however, the right to uncontaminated water has been upheld and liability imposed on those responsible for its pollution. One of the common methods of protecting ground water quality has been to declare activities which cause its contamination to be a nuisance[29]. In some cases the right to pure ground water has been viewed as absolute, with liability imposed on the party responsible for its degradation without regard to the reasonableness of his activity or the care with which his operation is conducted[30]. It is interesting to note that acceptance of the strict liability concept in the law is increasing[31], a fact which generally enhances the right of an injured party to recover from those responsible.

One of the most difficult aspects of ground water pollution cases is the problem of establishing a cause-and-effect relationship between the polluted water supply and the alleged source of the pollution. Since direct evidence as to the source is often impossible to obtain, the courts have often accepted indirect proof based on circumstantial evidence in such cases. It is difficult to generalize with regard to the type of evidence which will be sufficient in a given case, but factors that have been considered include the proximity of the alleged source, the existence of other possible sources, the time relationship between the alleged pollution-causing activity and the injury, and the capability of the suspected source to pollute[32]. Because of the fact that ground water contamination suits are often initiated and decided with relatively imprecise data, it would appear desirable that provisions for monitoring ground water quality include data collection prior to operation of the site as a treatment facility such that background data will be available for comparative purposes in the event that complaints of quality degradation arise.

In addition to the protection of quality, water law will also protect the landowner from interferences with ground water levels which produce injury. Underdrains are a method of mitigating both the qualitative and quantitative effects on ground water, but to date they have not been extensively used. A survey by the American Public Works Association indicated that only 4.9% of the 122 disposal fields examined had underdrains. Designers of the systems relied on evaporation, plant transpiration, and ground water recharge to take up the flow[33]. It must be recognized that under certain circumstances the increased subsurface flow may reach adjoining lands and so raise the ground water level that agricultural or residential development of the property is adversely affected.

Liability for artificially raising ground water levels has been imposed by the courts. For example, there have been several cases concerning damage to adjoining property as the result of the construction of reservoirs from which there was subsurface leakage. In an Ohio case[34], the plaintiff proved to the satisfaction of the court that a nearby impoundment had caused water to flow, ooze, percolate, or seep into the land of the plaintiff, thereby rendering it sour, wet, or swampy, and permanently unsuitable for use as farm land or for subdivision and sale. The plaintiff based its claim for damages on the concept that defendant had committed a "trespass"--a physical invasion of the plaintiff's property which destroyed the latter's right to the enjoyment thereof. The court held that liability was absolute without regard to whether the defendant had used due care in the construction and maintenance of the dam and reservoir.

An earlier Florida case[35] had a very similar fact situation. The court found in favor of the landowner injured by the subsurface water on the basis that damming a stream and causing an increase in ground water levels such that injury was produced was unreasonable. This case also addressed the question of whether a defendant can escape liability when the dam was built under authority granted by the United States Government. An analogy could exist to the case of land disposal since the federal government is likely to provide much of the funds for the project and in so doing gives its implied consent to the project. The court in the reservoir case held that authority to erect the dam did not justify an unreasonable use of the property as it affects the rights of others.

SURFACE WATER

Rights to water in its natural state are considered and dealt with as real property in almost all jurisdictions. Water differs, however, from other real property in that it is the use of the water and not the water itself which is the subject of property rights. This qualification or condition is found in each of the two major water doctrines prevalent in the United States--riparian and appropriative. The riparian doctrine recognizes a property right in the water only when the use being made is reasonable in terms of others having an equal right. Under the appropriative doctrine, a property right does not arise until the water has been put to beneficial use. So strong is this requirement that it is often said that beneficial use shall be the basis, the measure, and the limit of the right to the use of water. There is general agreement that "beneficial use" and "reasonable use" both carry economic as well as legal connotations.

The application of municipal sludges and effluents to land actually constitutes an "add on" after water has been applied to beneficial use by a local unit of government. Although this recycling has most of the attributes of irrigation, the rate of application and other parameters are defined not in terms of optimum irrigation but of obtaining improved water quality. Improved water quality may well be accepted as a national goal or objective, but unresolved is the question of whether it can or should be obtained at the expense of property rights in water as established by state law. The question must be asked as to whether this recycling of wastewater is a beneficial use or a reasonable one under our current water law doctrines.

The general rule in those states following the appropriative doctrine is that a water use once established will remain substantially unchanged with regard to its effects on other appropriators. The right in the use of water includes the right to change the place, nature, and means of use as well as the point of diversion, but the right to change is qualified in that it may not be exercised to the detriment of other appropriators, including those with junior rights[36]. Thus a conflict arises where a proposed modification in use involves a change from non-consumptive use to consumptive use which will deplete streamflow. Land application of municipal wastewater would appear to fall into this category of change since irrigation consumes more water than does traditional municipal use alone. In fact, the appropriator located immediately downstream from the prior point of waste discharge may be denied all use of the previously existing return flow.

Although conversion from conventional treatment to land application appears to be in contradiction to a basic principle of appropriation, limited precedent in this area does not indicate that the interests of junior appropriators will necessarily be protected. A 1972 Colorado case[37] concerning a change in point of effluent discharge is relevant to this issue. In this case, the court draws a distinction between a change in the point of diversion and a change in the point of effluent return. The court acknowledges that an appropriator may not change the point of diversion except upon conditions which eliminate injury to other appropriators, but goes on to say that changes in points of return of wastewater are not governed by the same rules. It is held that there is no vested right in downstream appropriators to the maintenance of the same point of return of irrigation wastewater, and in the absence of bad faith or arbitrary or unreasonable conduct, the same rule applies to sewage effluent from a municipality or sanitation district. The court does acknowledge that there may be instances in which a change of the point of return may be enjoined, but the fact situation of this case is not one of them. The case has a strong dissent which argues that the language of the court in previous cases is not limited to situations involving a change in point of diversion but that the court has set forth guidelines applicable to any change which an appropriator might make in the mode or manner of use of his water right. Nevertheless, the majority holding in the case represents a viewpoint that may permit land application operations to function without accountability to those whose water rights are adversely affected thereby.

The riparian doctrine is based on the simple premise that he who owns land traversed by or bordering a flowing stream may make a reasonable use of the waters thereof so long as such use is reasonable in the light of others having a similar right. After making a reasonable and proper use of the water for his own purposes, a riparian generally must return all the surplus water to its natural channel before it reaches the land of lower riparian owners[38]. Because of the amount of land involved in land disposal, it would seem unlikely that land to be used for disposal would be so situated that the return flows would naturally reach the stream of origin at a point above the land of all lower riparians. Therefore, land application is likely to be in violation of the rights of other water users on the stream.

The situation is further complicated where the return flows from land application ultimately find their way to a stream different from their origin. Interbasin transfers are generally not recognized under the riparian doctrine. Of course, a cause of action in cases of interbasin transfers or other violations of riparian rights does not arise in the absence of actual injury to the rights of a riparian landowner[39]. Therefore, legal obstacles to land treatment operations may not exist where a water surplus exists. In the absence of a surplus, the water rights of landowners on the stream may become a significant constraint. If these water rights are given full protection by the courts, land application may be excluded in some riparian situations without special provisions for acquiring the water rights of those affected or returning the effluent after land treatment to a point above the lower riparian owners.

The riparian doctrine is basically concerned with the interests of landowners who hold property adjacent to a stream by protecting the quantity and quality of the flow. This procedure automatically results in a substantial measure of protection to the natural water course itself, its scenic attributes, and many instream uses such as recreation and fish and wildlife habitat. More liberal interpretation by the courts on such questions as "standing to sue" and "class actions" greatly expand the potential for liability where the recycling of effluents on lands significantly modify the regimen of the stream. It thus becomes evident that surface water problems must be acknowledged and effectively dealt with in the design of land disposal systems to be located in the East.

SUMMARY AND CONCLUSION

In conclusion, it is obvious that consideration of potential legal constraints must be an integral part of the planning and design of systems for applying wastewater to land for the production of food and fiber. Because of the nature of these constraints, they cannot be treated as final design considerations but must be kept in mind from the initiation of planning. Ultimate feasibility of a given installation may well be dependent on these factors, and basic aspects of the physical design may be controlled thereby. Failure to give complete and timely recognition to these constraints is likely to produce problems during the operational stage and result in inefficiency and frustration in the application of the concept. Only when these constraints are fully incorporated into the total design framework can the potential of this technique be effectively realized.

Footnotes

1. Water Pollution Control Act of 1948, 62 Stat. 1155 (1948).

2. Federal Water Pollution Control Act of 1956, 70 Stat. 498 (1956).

3. Federal Water Pollution Control Act Amendments of 1972, 86 Stat. 866 (1972).

4. *Id*., sec. 301(b)(1)(B).

5. *Id*., sec. 201(g)1, 212(2)(A).

6. *Id*., sec. 201(d)(1)

7. 40 CFR Part 35, Appendix A(e)(1).

8. Federal Water Pollution Control Act Amendments of 1972, 86 Stat. 866 (1972), sec. 402(b).

9. Center for the Study of Federalism, *Green Land--Clean Streams: The Beneficial Use of Waste Water Through Land Treatment*, Philadelphia, Temple University, 1972, p. 226.

10. Environmental Protection Agency, *Survey of Facilities Using Land Application of Wastewater*, Washington, Environmental Protection Agency, 1973, p. 99.

11. *California Administrative Code*, Title 23, Waters, Chapter 3, Subchapter 15.

12. Idaho Board of Environmental and Community Services, "Rules and Regulations for the Establishment of Standards of Water Quality and for Wastewater Treatment Requirements for Waters of the State of Idaho," sec. XI, 1973.

13. *Lees* v. *Sampson Land Co*., 92A. 2d 692 (Pa. 1952).

14. 101 C.J.S. Zoning sec. 135.

15. James A. Ballentine, *Ballentine's Law Dictionary*, Rochester, N.Y., The Lawyers Co-operative Publishing Company, 1969, p. 530, 1012.

16. *O'Brien* v. *Town of Greenburgh*, 268 N.Y.S. 173 (N.Y. 1933), *aff'd*. 195 N.E. 210(N.Y. 1935).

17. *Kedroff* v. *Town of Springfield*, 256A. 2d 457 (Vt. 1969).

18. *St. Louis County* v. *City of Manchester*, 360 S.W. 2d 638 (Mo.1962); *see also Jefferson County* v. *City of Birmingham*, 55 So. 2d 196 (Ala. 1951).

19. *City of Scottsdale* v. *Municipal Cout of Tempe*, 368 P. 2d 637 (Ariz. 1962); *see also City of Des Plaines* v. *Metropolitan Sanitary District of Greater Chicago*, 268 N.E. 2d 428 (Ill. 1971); *People Ex. Rel. Scott* v. *North Shore Sanitary Dist*. 270 N.E. 2d 133 (Ill. 1971).

20. *See, e.g. Bader* v. *Iowa Metropolitan Sewer Co.*, 178 N.W. 2d 305 (Iowa 1970); *Ward* v. *Hampden Township*, 271 A. 2d 895 (Pa. 1970).

21. *See*, *e.g.*, *Fields Sewerage Co*. v. *Bishop*, 30 S.W. 2d 412 (Tex. 1930).

22. *See*, *e.g.*, *City of Marlin* v. *Criswell*, 293 S.W. 910 (Tex. 1927).

23. *See*, *e.g.*, *City of Temple* v. *Mitchell*, 180 S.W. 2d 959 (Tex. 1944).

24. *See*, *e.g.*, *Aguayo* v. *Village of Chama*, 449 P. 2d 331 (N.M. 1969).

25. *Ryan* v. *City of Emmetsburg*, 4 N.W. 2d 435 (Iowa 1942).

26. *See*, *e.g.*, *Hulbert* v. *California Portland Cement Co.*, 118 P. 928 (Cal. 1911); *Mitchell* v. *Hines*, 9 N.W. 2d 547 (Mich. 1943).

27. *State ex. rel. Knight* v. *City of Miami*, 53 So. 2d 636 (Fla. 1951).

28. *See*, *e.g.*, *North East Coal Co.* v. *Hayes*, 51 S.W. 2d 960 (Ky. 1932); *Rose* v. *Socony Vacuum Corp.* 173 Atl. 627 (R.I. 1934).

29. *See*, *e.g.*, *Love* v. *Nashville Agricultural and Normal Institute*, 304 (Tenn. 1922); *Swift and Co.* v. *Peoples Coal and Oil Co*., 186 Atl. 629 (Conn. 1936).

30. *See*, *e.g.*, *Berger* v. *Minneapolis Gaslight Co.*, 62 N.W. 336 (Minn. 1895); *Berry* v. *Shell Petroleum Co.*, 33 P. 2d 953 (Kan. 1934).

31 W. Prosser, *Handbook of the Law of Torts*, St. Paul, Minn., West Publishing Co., 1971, p. 509.

32. *See*, *e.g.*, *Hall* v. *Galey*, 271 P. 319 (Kan. 1928); *Bumbarger* v. *Walker*, 164 A. 2d 144 (Pa. Super. Ct. 1960); *Jackson* v. *U.S. Pipeline Co.*, 141 A. 165 (Pa. 1937).

33. Environmental Protection Agency, op. cit, p. 73.

34. City of Borbenton v. Miksch, 190 N.E. 387 (Ohio 1934).

35. Cason v. Florida Power Co., 76 So. 534 (Fla. 1917).

36. Robert Emmet Clark, Ed., Waters and Water Rights, Vol. 5, Indianapolis, The Allen Smith Company, 1972, p. 428.

37. Metropolitan Denver Sewage Disposal Dist. No. 1 v. Farmers Reservoir and Irrigation Co., 499 P. 2d 1190 (Colo. 1972).

38. 93 C.J.S. Waters sec. 62.

39. See, e.g., Virginia Hot Springs Co. v. Hoover, 130 S.E. 408 (Va. 1925); Stratton v. Mt. Hermon Boys' School, 103 N.E. 87 (Mass. 1913).

SOCIAL, POLITICAL, REGULATORY AND MARKETING PROBLEMS OF MARINE WASTE-FOOD RECYCLING SYSTEMS*

John E. Huguenin
Judy T. Kildow

INTRODUCTION

The coastal areas of the United States are receiving ever mounting public attention due to their increasingly apparent importance to the national well being. The pressures emanate from the increasing demands on the coastal waters and exploitation of their contained resources combined with the increasing risk of destroying some of these very resources by pollution due to the presence and activities of large adjacent populations. Thus, at times, coastal zone resource exploitation and resource conservation appear to be conflicting objectives. But need they be conflicting?

It has become customary to consider any and all of the waste products of our society as pollutants, rather than resources, and to view their discharge into the environment as a form of pollution. However, the nature and ecological effects of man's wastes are so highly variable that their common designation and implied common impact on the ecosystem may be a concept both simplistic and misleading.

There are at least some marine waste-food recycle concepts that from a scientific and technical viewpoint seem very promising (see Table 1). There is also currently considerable effort being expended both nationally and internationally in the science and technology of using societies' wastes as a resource in marine aquaculture. This does not mean that there are not many serious obstacles to be overcome before some of the proposed systems can be considered ready for large scale operational use. Others, on the other hand, are already being used on a large scale both intentionally and unintentionally in sea food production. Agricultural and fishery processing wastes are commonly used in formulated diets for animals both terrestrial and aquatic, possibly without full understanding of some of the potential

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Table 1. Uses of "Wastes" in Marine Aquaculture

Waste	Possible Uses	Possible Benefits	Comments
Thermal effluents from power plants	Provide water temperature control capability	Up to 500% (depending on circumstances) increase in yearly production due to water temperature control	Greal deal of current research activity and commercial interest
Secondarily treated domestic sewage	As fertilizer to grow marine plants	Large "free" nutrient source at base of marine food chain and also providing a form of tertiary sewage treatment	Current research promising - many possible variations and applications
Agricultural and fishery processing wastes	As components in formulation of feeds	Reduced aquaculture feed costs	Already common practice
Compacted solid wastes	As construction material for artificial reefs	Providing habitats to enable denser concentrations of marine animals	Small scale research in progress - Not a preferred material for reef construction
Sewage sludge	As fertilizer or as input for production of protein to be used in animal feeds	Reduced aquaculture feed costs	Small scale research in progress

risks involved. As an example, the over 600 state and federal fish hatcheries in the U.S. alone utilize more than 41 million pounds of fish food yearly, with almost 23 million pounds of this being organic wastes (Hinshaw, 1973)[1]. The potential for the beneficial use of power plant waste heat is widely recognized and is one of the aspects of waste utilization currently receiving the most attention (Mathur and Stewart, 1970[2]; Yarosh, 1973[3]). The use of algae in processing domestic sewage is accepted practice in many parts of the United States. Current efforts are in progress to extend these practices to produce useful food organisms in a marine environment (Ryther, Dunstan, Tenore and Huguenin, 1972[4]; Allen, Conversano and Colwell, 1972[5]). The use of sewage ponds to grow aquatic food animals is not at all a new concept (Allen, 1970[6]). Sewage products which are dumped in large quantities into our coastal waters are certainly, at least in part, responsible for the high sea food yields from some of our estuaries (Ryther, 1971[7]). However, this increased productivity due to fertilization with wastes is often unintentional, generally not recognized and does not lend itself to management. The risks are nevertheless present.

Time and research and development effort can be expected to make some of these concepts both technically feasible and economically attractive. It is not clear, however, whether even strong technical and economic justifications are sufficient to assure the application and exploitation of new developments by our society. Apparently not, for there are other considerations and constraints. These obstacles must be systematically and rationally analyzed before considerable resources are expended to develop technically sound systems only to find them useless due to critical legal, socio-political or psychological factors.

ADVOCATES AND BASIS FOR OPPOSITION

Marine aquaculture, in general, is already constrained by a host of institutional factors. Previous efforts dealing with these seem to have concentrated on land and water rights (Henry, 1971[8]; Kane, 1970[9]; Shields, 1970[10]). However, there are many other problems upon which effective opposition might focus (see Table 2). The use of resources currently considered wastes in the production of marine foods may intensify institutional scrutiny of such operations, due to both the nature of the wastes and the increased visibility of the projects. This is further complicated by the fact that marine aquaculture does not have any formally organized interest groups to lobby for its interests and for its rights in the heavily contested coastal zone. In contrast there exist many well established and powerful groups which could form effective opposition if they were to perceive marine aquaculture as a threat to their interests in coastal and estuarine areas. There is no lack of vulnerable points for opponents to exploit. However, this is counterbalanced by the growing awareness of shortages

Table 2. Potential Sources of Opposition to Aquaculture

- Illegal use of chemicals by aquaculturists.
- Local and state conservation and game regulations.
- Competition for sites from alternate users.
- Industrial fears of more pressures to reduce water pollution.
- Secondary products flooding small markets.
- Countermeasures to prevent poaching and vandalism.
- Pollution potential of large cultures.
- Alterations to coastal areas.
- Transplants of organisms both planned and unintentional.
- Characteristics of occasional disasters.
- Presence in cultures of nuisance diseases and parasites.
- Concerns over potential hazards to public health.

and the need to effectively manage our coastal resources. The fact that there are no organized advocates at present is a partial advantage in that attitudes of potential political oppositions are still flexible and there are no commitments to past mistakes. Waste food systems in particular have unique possibilities for a broad appeal to many different elements of our society not possible with straight commercial aquaculture. Many of the potential opposition groups may, if care is taken, become strong supporters. This is due to the multi-objective possibilities of such systems which combine pollution control, growth of food and the use of otherwise wasted "resources". This provides both a strength and a weakness. Making judgements in areas where objectives conflict may prove troublesome. In addition, there is the danger that with more research, what now appear to be generally compatible objectives may prove to be incompatible. The broad appeal may also lead to a premature "push" for acceptance resulting in opposition based on scientific and technical issues which could easily be diffused with more thorough research. Many of these potential issues involve areas in which so little research has been done that it is not even known with certainty whether or not substantial problems in fact exist. This is especially true for marine waste-food recycle concepts, due to the many potential and unresolved questions particularly in the areas of public health and quality assurance. Combining two such critical human activities as food production and the removal of wastes can very easily produce opposition based on fear resulting from uncertainty.

PREREQUISITES FOR ACCEPTANCE

There are several prerequisites before societal acceptance of waste-food or water reuse systems for general use is a realistic possibility. First, all legitimate public health concerns must be adequately assessed and resolved (Shuval and Gruener, 1973[11]). Two examples where sewage products are involved stand out. One is the problem of enteric virus (Ramas, 1970[12]; Metcalf, Vaughn and Stiles, 1970[13]), and the other is the possible presence of known carcinogenic chemicals in the input wastes. There are also unknowns involving the transmittal and concentration of various "pollutants" through complex food chains which include man, and the long term effects of these processes. Systems must be developed to reduce or circumvent the high risk areas, and adequate public health safeguards must be provided. If this is not done, there is very little chance for societal acceptance unless the perceived needs are so imperative and pressing that high risks are warranted. The inverse is also true. Definite markets must either exist or be created for even low risk waste-food systems to receive societal and regulatory acceptance. These markets may be for direct domestic use or more indirect, such as for the benefit derived from exports or even for non-material advantages, such as for environmental enhancement. But these needs must be perceived, and believed by at least a portion of our society for any hope of favorable actions. The very existence of this conference indicates that, to some degree, this is already fact.

Given a latent but generally perceived societal need, potential markets, and promising waste food system possibilities, at least some favorable public policy decisions seem a prerequisite for the establishment of any full scale demonstration projects. At a minimum, it would require some indications that if the systems meet some specified performance levels the outputs would be approved for public use. It is important to remember that most systems at this point are not completely proven, and that uncertainties also exist about both performance at full scale and over economic viability. These uncertainities can remain a basis for skepticism and opposition in spite of successful research efforts. The economics, in particular, of many proposed aquaculture systems are open to question, because almost all the published research has been done in very small laboratory experiments and the little data that exists for large cultures is generally proprietary information. In addition, pilot plant facilities are usually designed to research a spectrum of possible concept variations, operating conditions and management approaches. As a consequence, they are usually much smaller than the ultimate systems, since the flexibility required for their continually changing experiments and from their physical plants would be cost prohibitive at full scale. Therefore the operations and economics of such research facilities, while very useful, have limitations when attempts are made to directly apply these experiences to large systems designed for routine use under closely defined operating conditions. Thus, these uncertainties can be fully removed only with the design, construction and operation of full scale demonstration systems, which need a favorable public policy environment to justify the tremendous efforts and expenses involved. This applied development stage may be the weak link in aquacultural development (Trimble, 1972[14]) due to the current lack of government support and to the high risks and insufficient incentives for private industry. However, it must be emphasized that small scale laboratory testing and sub-scale pilot plant facilities are an essential prerequisite for providing the needed design information for full scale demonstrations. There are, however, pressures to speed the development of promising waste-food systems to large scale applications. Prematurely committed demonstration projects that fail, due to inadequate preparation, pose a real danger in that they may discredit basically sound solutions.

While determining "safe" levels of contaminants in foods poses an extremely complex scientific, economic and political dilemma, some general guidelines are required. The current lack of realistic quantitative standards is a major problem. A criterion that might be acceptable to all is that the waste-grown sea food outputs be, in all respects, as good as or better than the same products found on the market from natural sources. This could solve the problem of providing design criteria and "acceptable" goals for the development of future systems. Also, while not necessarily easy, it is probably not as restrictive to the aquaculturist as it may at first appear. It must be remembered that the aquaculture output is a managed product and inherently susceptible to much improved quality control comparable to "wild" products, which in many cases have not even been carefully monitored or analyzed. When analyzed, the latter may be found to have surprisingly high levels of some "pollutant" whose presence may not even be related to any activities of man (e.g., mercury in swordfish and other fish). Requiring completely "zero additions" of any pollutant, such as is done for "known" carcinogenic chemicals in applying the Delaney amendment to the Food, Drug and Cosmetic Act of 1938, while allowing "non harmful" quantities of the same substances if found "naturally", is certainly an unrealistic double standard which could easily preclude the hope and potential promised to mankind by aquaculture, whether it uses wastes in the process or not.

CHOICES

What sort of policy options do the marine waste-food advocates have? It is clear that the policies adopted by advocates as well as their decisions in the fields of marketing and system design will affect the types and severity of the opposition. A major option is in the choice of the initial outputs which are somewhat independent of the long term objectives of the projects and seem a flexible variable based on what is currently known about aquaculture food webs. Thus the initial outputs may not be marine foods for human consumption but rather products for education, research, bait or only as food additives. It seems probable that societal acceptance will be inversely proportional to the intimacy of the contact with the product and its waste input (Bruvold and Ward, 1972[15]). Thus, the probability of successful societal adaption might be increased by initially concentrating on other than direct food outputs. Early systems should, as much as possible, hedge against uncertainties by maintaining the capability of producing a spectrum of different products. Another option, assuming a direct food output is desired, is whether to produce relatively small quantities of high quality, high value "luxury" sea foods or for large quantity "protein". Production oriented aquaculture operations in both developed and less developed countries have consistently and without any notable exceptions attempted to produce "premium" foods. In the less developed countries this has often been primarily for export although local food shortages may exist. In addition, experience has shown that cultured products almost invariably receive premium prices over the same product from natural sources. Thus, if economic criteria alone are used, any large scale aquaculture operation is almost certain to involve high unit value products. However, waste food systems are much more likely, due to their association with public utilities, potential health hazards and involvement with current public concerns, to have decision criteria that are not based solely on marketplace economics.

Another major policy choice to be made by waste food advocates is whether to go for a high degree of publicity, presumably favorable, or adopt a policy of secrecy. Obviously, this decision in many cases may not be completely within the powers of this group. Aquaculture, in general, has been receiving a great deal of publicity, almost all very favorable and also, by and large, unrealistically optimistic. On the other hand, actual commercial aquaculture ventures have generally been carried out with a very minimum of publicity. This may be due both to the proprietary nature of the operations and their vulnerability to opposition because of some of their practices, as well as to the generally unfavorable legal/political environment. In addition, successful aquaculturists have generally been well integrated with their rural power structures, have usually conducted their business completely on private land and have operated on a scale small enough not to form a focus for opposition. In contrast, well organized efforts to set up large commercial aquaculture operations needing substantial capital and specialized expertise have often met failure, due in part to their planned scale of operations and association with "outside interests".

A noticeable exception to this low profile policy have been operations carried out in cooperation with power plants. However, most of these operations have been experimentally oriented rather than direct commercial attempts. In the few commercial cases, publicity has been due to the power company's desires for favorable public relations involving their thermal discharges rather than any change of policy on the part of aquaculturists. In addition, while these few operations have not been opposed, they have been relatively small in scale with little impact and they have been surrounded with an aura of research and experimentation. Thus, reactions to these operations are not necessarily indicative of the reception to be experienced by large scale operational systems. It may not be reasonable to assume that the past history of secret or semi-secret aquaculture operations is a realistic possibility for future large aquaculture systems, especially if wastes are used. A policy of secrecy for the planning and development of a large commercial system might be realistic only if it were known with confidence that the use of wastes so lowered the production costs and that the associated technical risks and probabilities of unpleasant external effects were so low that the risks involved with presenting society with a "fait accompli" would be justified.

CONSUMER ACCEPTANCE

Assuming that all other problems were solved, there is still the question of consumer acceptance of waste-grown sea foods. There are several important variables involved. Probably the most critical is the degree and circumstances surrounding the differentiation of waste grown marine foods from other sources of the same products, as may be required by regulatory agencies. This is a problem mostly in the fields of packaging, labelling and advertisement. It is anticipated, but not yet proven, that the products will look, taste and smell the same as their managed counterparts which are not grown with "waste" inputs. This is obviously a critical assumption. If little or no differentiation is required, there is in all likelihood no marketing problem. Past rulings of the Food and Drug Administration and the Federal Trade Commission, suggest assuming the worst case conditions.

Assuming that distinct labelling will be required, to what extent does a consumer's feelings of repugnance for wastes, which are known to be acquired rather than innate or physiological, carry over to food products grown with such materials? Unfortunately, there does not appear to be any available research directly dealing with this interesting question. However, if the fishing aspects of the Santee Project (Merrel et al., 1967[16]) and the common use of wastes in agriculture are any indication, this associative link may either not always occur, or can be nullified, even when the physical facts are clearly known. There is also considerable literature on public attitudes towards the closely allied concept of using reclaimed water (Bruvold and Ward, 1970[17]; Bruvold, 1971[18]; Bruvold, 1972a[19]; Bruvold, 1972b[20]; Gallop Poll, 1973[21]). Small scale surveying at M.I.T. (to be published) has shown good correlation of this data with consumer attitudes towards seafoods grown with waste inputs. Even studies on attitudes toward irradiated fish (Yankelovich, 1966[22]) and on fluoridation (Sapolsky, 1968[23]) show surprising similarities. However, while it is relatively certain that a large segment of the public would not oppose the use of wastes in marine aquaculture, there is little information on the numbers or degree of activism of either strong supporters or strong opponents. This is a critical void, since small vocal minorities have often swayed public opinion in similar situations as demonstrated by the history of local fluoridation decisions (Sapolsky, 1968[23]).

It must be remembered that the fact that aquacultural products are cultured and managed, presumably under adequate controls, is a strong selling point and one exploited by all present commercial aquaculture operations. The available literature indicates that those individuals who approve of the use of potentially objectionable inputs do so under the assumption that such systems, if established, would have adequate controls. These feelings of assured quality may be strong enough for some consumers to make waste-food products preferred over sea foods of "unknown" quality from other sources. In all studies, the major sources of consumer opposition have been uncertainties and intuitive feelings concerning uncleanliness, impurities and lack of public health safeguards. Thus, the public's confidence in the safeguards and quality control of the output products may well be the single most important factor in consumer acceptance of waste grown foods. Obviously one incident of bad publicity involving the quality of the products could be extremely damaging.

These problems highlight the importance of deficiencies and inadequacies in monitoring and quality control technology. While very sensitive analytic methods have been developed for detecting most pollutants and contaminants, these methods, in many cases, are not adequate for long term continuous or quick reaction monitoring in a biologically active seawater environment, as would be required for applications in operational systems. Research and development effort is especially needed to apply and modify current laboratory methods and equipment to produce reliable and practical monitoring systems.

CONCLUSION

In spite of the many problems (see Table 3), the future for marine waste-food recycling systems is very promising. In fact, a good case could be made that such systems are already operating unintentionally on a large scale in some regions of our coastal zone. While at the present time the recycling of wastes into our food supplies is neither planned nor managed, this is sure to change. At some point, the increasing demands on the coastal zone for both waste disposal and food production can be safely met only by acquiring some degree of control over the systems involved. Only in managed situations can the risks be controlled and production increased. Our real choice is whether this is to be done methodically or haphazardly. Due to the required long lead times for planning, research and development, the time for choice may be now.

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Table 3. Problem Areas Requiring More Effort

- Resolving potential public health hazards.
- Establishment of realistic "acceptable" levels for "pollutants".
- Better understanding of food chain dynamics.
- Applying existing technology to monitoring and control problems.
- Support for promising "pilot plant" and "demonstration" projects.
- Economic evaluations and comparisons with alternative approaches.
- Market testing of outputs for quality and acceptance.
- Development of public and private institutional infrastructure.

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RECYCLING FOR A PURPOSE-BUT FOR WHAT PURPOSE? A SOCIOLOGIST'S VIEW

by

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With the political and social unrest persisting in today's world, the public is beginning to question the very existence of many of the social institutions. One constantly hears statements such as: "There is no need to be concerned with the population problems, because as the population grows so will our level of technology and everything will be O.K.—besides, it's my *right* to have as many children as I want"; or "There is no real energy crisis, it's just the government and big oil companies trying to drive the price of gasoline up, the little man out and the customers off their backs. There is enough energy for everyone." In essence, the people do not know which line of rhetoric to accept and when a true social issue is presented they view it with a jaundice eye. A key question which the physical scientific community must address is "How do we determine the existing public opinion toward a topic, and once known, how do we alter those opinions to facilitate a social acceptance of the problems in order to allow a joining of efforts on the parts of the scientific community and the public community to abate the issue?"

Turner and Killian have suggested that

> "At times...problems arise for which tradition provides no clear-cut solution. An issue is created and a public arises. Public opinion is formed and reformed, often with startling shifts, until the issue disappears. The major norms of a society as embodied in its institutions persist for long periods in spite of varying degrees of dissatisfaction with them. But periodically social movements develop which culminate an institutional change and cultural revision."[1]

WHAT IS PUBLIC OPINION?

In order to evaluate the attitudes of the public, one must first understand that public opinion is the "...*effective expression of the public....* [realizing that] the public...is a dispersed group of people interested in and divided about an issue, engaged in discussion of the issue, with a view to registering a collective opinion which is expressed to affect the course of action of some group or individual."[2] Of course there are many definitions of public opinion but for purposes of this paper the above suffices.

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[1] Turner, Ralph H. and Lewis M. Killian. Collective Behavior. Engelwood Cliffs, Prentice-Hall, Inc., 1957, p. 3.

Consequently, one may interrelate public opinion and individual opinion understanding that the feeling, attitudes and actions of the group are attributable to the phenomena of the individual.[3] "Public opinion then becomes a form of group thinking, and the process bears more than an analagous relation to the individual's 'complete act of thought.'"[4]

In order then to understand the attitudes of the group, one must measure, in some form or another, the attitudes of the individual toward the question under consideration. Peter Kelvin explained that

> "...an attitude has at least two components: there is the 'object' of the attitude or, rather, what the individual knows or believes about it; and there is his feeling towards it, which is the basis for how he evaluates it. If we... look at the meaning we give 'attitude' in everyday language, we find a third component, by implication related to behaviour."[5]

To examine the three components of an attitude from another perspective, "Feelings are often referred to as the ***affective*** components, thoughts as the ***cognitive*** component, and predispositions to act as the ***behavioral*** component."[6]

To the layman the behavioral and cognitive components of attitudes appear to be the most obvious factors for measurement; however, the outward displays of personality do not necessary coexist with the inward thought patterns and ***vice versa***. To explain this in another fashion: When a person discriminates either positively or negatively toward another, it does not mean there is a coexisting positive or negative prejudice acting in conjunction with the former. Therefore, "From the standpoint of research technique, observable behavior is in fact very difficult to measure, and the actual behavior observed may have relatively little to do with basic beliefs and feelings."[7] Consequently, the focus of attitude research is toward the affective component. In essence, attitude measurement is the measurement of feelings and opinions directed toward an object or issue.

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[2] Ibid. p. 219.

[3] Lunberg, George A. Public Opinion from a Behavioristic Viewpoint. American Journal of Sociology. 36:387-405, November 1930.

[4] Clark, Carroll D. The Concept of the Public. Southwestern Social Science Quarterly. 13:311-320, March 1933.

[5] Kelvin, Peter. The Bases of Social Behaviour--An Approach in Terms of Order and Value. Great Britain, Holt, Rinehart and Winston, 1970, p. 40.

[6] Secord, P. F. and C. F. Backman. Social Psychology. New York, McGraw-Hill, 1964, p. 97.

This phenomena gives rise to one of the major difficulties prevailing between the physical sciences and the social sciences. How does one measure an ambiguous concept that has no mass or physical component? To do this the social scientist has had to essentially rely on a method of ordering or scaling.

ATTITUDE MEASUREMENT FOR THE FACILITATION OF CHANGE

Predictivity must necessarily be based upon extrapolative conditions and the facilitation of attitude changes toward ecological phenomena must be accomplished by congruence between theory and practice. In order to accommodate this task the community must be preconditioned to accept the feasible technological solutions to perceived problems. The principle of attitude measurement or scaling "...is a form of planned collection of data for the purpose of description or prediction as a guide to action or for the purpose of analyzing the relationship between certain variables...."[8] If one is to understand the level of thinking of an individual or public toward a topic (e.g., how a public feels toward recycled products), one must develop an instrument of measurement appropriate for the specific task. Once this instrument has been developed, the data collected and analyzed, the social researcher will then be able to formatively design a technique which will be useful in redirecting the public opinion toward a more positive understanding of the concept.

To change the attitudes of a community or society is not an effortless task, as Thorstein Veblen warned, "Institutions are products of the past process, are adapted to past circumstances, and are therefore never in full accord with the requirements of the present."[9] Lewis A. Coser further examined Veblen's theory as

> "...a new technology erodes vested ideas, overcomes vested interests, and reshapes institutions in accord with its own needs. But this process may take considerable time, and in that time lag--when, for example, an industrial society

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[7] Kelvin, p. 40.

[8] Oppenheim, A. N. Questionnaire Design and Attitude Measurement. New York, Basic Books, Inc., 1966, p. 1.

[9] Veblen, Thorstein. The Theory of the Leisure Class. New York, Modern Library, 1937, p. 191.

> is still governed by legal and moral rules dating from the handicraft era--society suffers from the waste that is brought about by the lack of correspondence between its institutions and its technology."[10]

The change of attitudes of a social system "...implies a change in the individual's order of values, associated with changes in his beliefs and behaviour; and in essence any change in the way in which the individual orders his environment naturally affects the predictions which he makes about it and the way in which he adjusts to it and manipulates it."[11] The ability of the individual to change to the new technologies will be in concordance with the instrumentation used to provide the special knowledge, the efficiency with which the communication or learning devices are utilized and the effectiveness or its organization.

To further extend one's understanding of this phenomena, Turner and Killian suggest that "Certain fundamental conditions will determine the effectiveness of attempts to manipulate any type of collectivity."[12] These fundamental conditions are:

> "(1) All effective influence obviously depends first of all on gaining *access* to the group to be influenced....
>
> (2) ...the receptiveness of the mass toward the proposed course of action or thought. In the mass, the receptiveness [meaning interests, motivations and understandings] is that of the individual, since individuals must decide and act....
>
> (3) ...the possibility of carrying out the proposed action....
>
> [And finally]
>
> (4) ...the inclination and opportunity to act, the recipient still makes some evaluation of the appeal itself and of the assumed source of the appeal."[13]

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[10] Coser, Lewis A. Masters of Sociological Thought--Ideas in Historical and Social Context. New York, Harcourt Brace Jovanovich, Inc., 1971, pp. 272-273.

[11] Kelvin, p. 59.

[12] Turner and Killian, p. 277.

[13] Ibid. pp. 277-295.

SUMMARY

In order to achieve a social change of attitude toward a new philosophy of life the social scientist will have to be familiar with the current thinking and trends being developed in the physical scientific community. The social scientist will have to evaluate the current level of thinking of the community, design a program to institute social change and implement these changes in order to bring the social acceptability of the community in line with the degree of current technical know-how. This will require a formidable amount of lead time, which heretofore has not been a luxury afforded the social scientist. In short, the apathetic attitude demonstrated by the general public and many decision-makers cannot be dispelled unless social and physical sciences merge toward a collective approach to a solution.

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PART 6 NEW OR INTEGRATED SYSTEMS

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THE MICHIGAN STATE UNIVERSITY WATER QUALITY MANAGEMENT PROGRAM[a]

by

T. G. Bahr,[b] R. C. Ball,[c] and H. A. Tanner[d]

INTRODUCTION

One of the Nation's greatest environmental questions is what to do with vast quantities of municipal wastewater. Conventional methods of wastewater treatment can contribute importantly to wastewater clean up but the discharge of even these wastes has created eutrophication of lakes and degradation of waterways through the nutrient materials that they contain even after advanced treatment. We are also becoming aware of perhaps an even more important problem. Many products used by man contain elements that represent scarce and diminishing resources or compounds that have a high energy demand in their manufacture. In an economy faced with both material and energy shortages it seems foolish to manufacture products at high cost to both of these, use them once and then discard them. But this is exactly how we handle plant nutrients. We mine our increasingly limited supplies of phosphorus and infuse our scarce fossil energy into the production of nitrogen fertilizers in order to sustain the food production that feeds the millions in our cities only to waste these elements into the nearest stream. This gross mismanagement coupled with requirements for high water and air quality and the difficulties of removing nutrients to levels acceptable to discharge into natural waters has prompted an extensive search for alternate methods.

It is well known that nutrients present in wastewater can be turned into the production of harvestable food and fiber if applied in appropriate amounts to the land or to the water. The quantity and schedule of application of wastewater to agricultural lands or to aquaculture systems to achieve an optimal level of food or fiber production and, at the same time, achieving a high level of water renovation is not well understood at this time. The number and depth of research studies in this area has been very limited and there are many important questions vital to human health, energy and resource conservation that have not been answered. We believe that maximum efficiency of research on these questions can be obtained from an integrated study of recycling systems incorporating the facets of both terrestrial and aquatic ecosystems. The plans, estimates and designs for functioning reuse and recycling systems cannot be based on concepts but must be reduced to reasonably accurate estimates based on experience gained with working model systems. Such systems will require a coordinated multi-disciplinary effort of considerable magnitude.

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[a] This program is supported by grants from the Ford Foundation, Rockefeller Foundation, Kresge Foundation, the U.S. Environmental Protection Agency, Office of Water Resources Research, and the State of Michigan.

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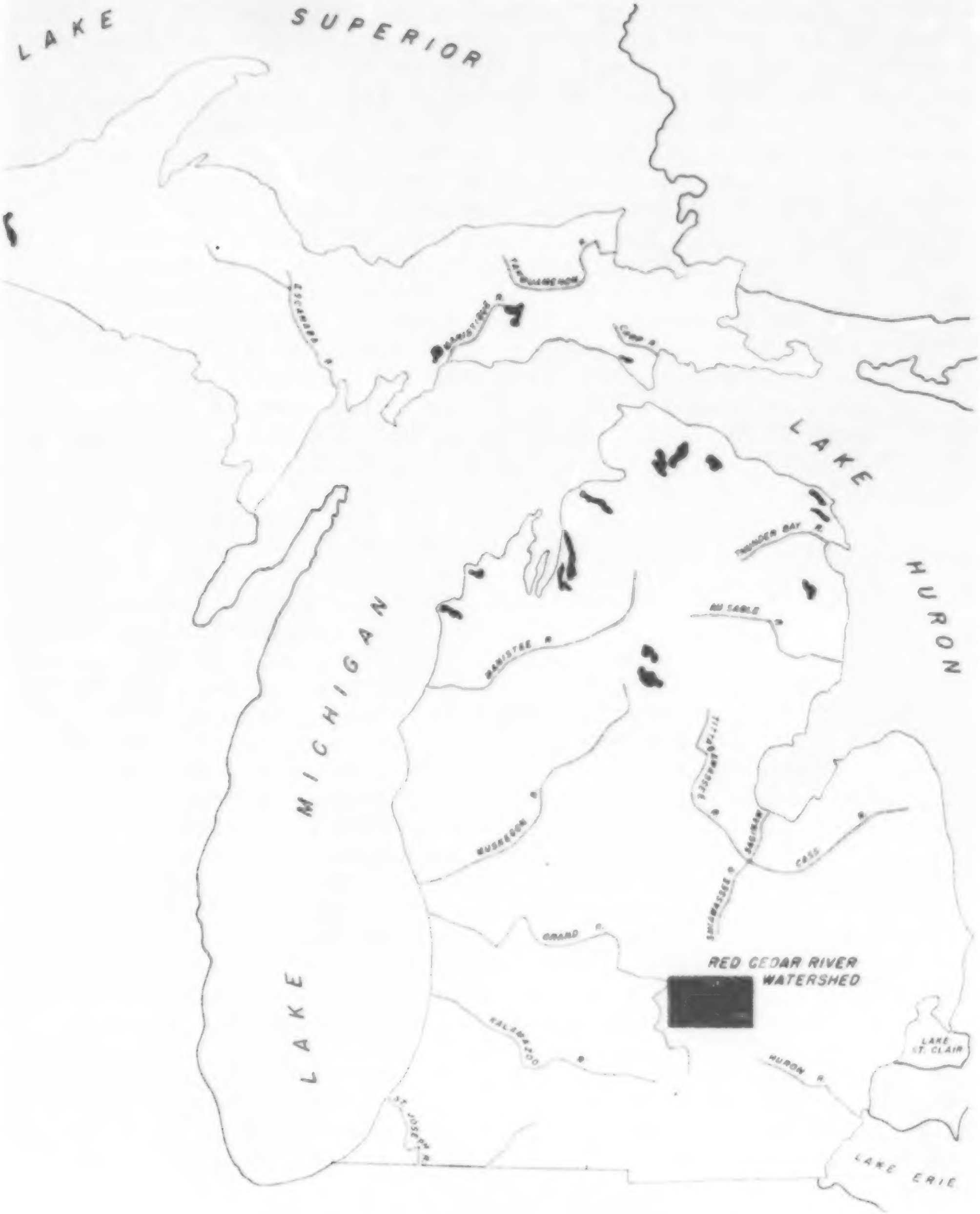
DEVELOPMENT OF THE PROJECT

Michigan is a state with vast water resources, bordering on four of the five Great Lakes. Our interests for the future are all closely tied to the discharge of wastes into these bodies of water. Almost without exception every pollutant that leaves a sewage disposal plant or runs off from our fields or urban areas will reach one of the Great Lakes. This can be seen in Figure 1 by examining the position of Michigan's major tributaries in relation to the bordering Great Lakes. Recent estimates by staff of the Institute of Water Research at Michigan State University place phosphorus (P) losses to Lakes Michigan and Erie from Michigan's lower pennensula at approximately 35 million pounds per year. The greater Detroit area in southeastern Michigan alone contributes nearly 20 million pounds per year, nearly all of which is from municipal or industrial sources (Bahr[1]). Michigan State University has a long history of research on pollutants and nutrients in streams (Ball and Bahr[2]) and a concern on the part of many of the staff for the well being of our water resources. The wastewater recycling project to be described in this paper was designed and built as a direct result of these concerns.

The development and administration of the project is a major part of the water programs of Michigan State University's Institute of Water Research located on the main campus. Prototype plans received financial support and approval to continue from the Michigan State University Board of Trustees in 1966. Since then we have been helped


LAKE SUPERIOR
ESCANABA R.
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TAHQUAMENON
CARP R.
LAKE HURON
THUNDER BAY R.
AU SABLE R.
LAKE MICHIGAN
MANISTEE R.
TITTABAWASSEE
MUSKEGON R.
SAGINAW
CASS R.
SHIAWASSEE R.
GRAND R.
RED CEDAR RIVER WATERSHED
KALAMAZOO R.
HURON R.
LAKE ST. CLAIR
ST. JOSEPH R.
LAKE ERIE

Figure 1. Prinicpal rivers in the State of Michigan and location of the Red Cedar River watershed, site of the Michigan State University wastewater recycling program.

and encouraged by many faculty, citizens and public officials in both state and federal agencies. Funding was eventually obtained from the Ford, Kresge, and Rockefeller Foundations and later from the U.S. Environmental Protection Agency and from the State of Michigan through its Clean Water Bonding Program. The project construction was completed during the Fall of 1973. Cost of construction, exclusive of land acquisition, totaled approximately $2.5 million.

PROJECT DESCRIPTION

The physical facility for the Michigan State University wastewater recycling project consists of four basic elements: (1) a conventional activated sludge sewage treatment plant; (2) a 4.5 mile transmission line; (3) a lake system; and (4) a land irrigation system. This system is schematically shown in Figure 2. The following is a more detailed discussion of the facility.

SEWAGE TREATMENT PLANT AND TRANSMISSION LINE

The East Lansing Sewage Treatment Plant services the City of East Lansing, Michigan State University with its 50,000 students and employees, and the adjoining community and represents the first element of our system. It is presently undergoing modification and enlargement to a capacity of 15,000,000 gallons per day. As an integral part of this modification the Michigan State University program will develop and operate a parallel unit within this plant that will have a capacity of 2,000,000 gallons per day. Our portion will differ from the total plant in that the effluent being received will not have pre-treatment for the partial chemical removal of phosphorus. The effluent (secondary or primary) from this subunit can be directed to a pumping station with a present capacity of 2,000,000 gallons per day and provisions for additional pumps to increase its capacity to 6,000,000 gallons per day. Flow will be transmitted through the second element of the system, a 21 inch concrete-asbestos pipeline which traverses 4.5 miles to the southern border of the Michigan State University campus and on to the 500 acre research site. Here it discharges into the first of four man-made lakes.

LAKE SYSTEM

The flow from the first lake is then by gravity through each of the other lakes and to a control building and pump house servicing the adjacent spray irrigation site. The lakes have a total surface area of 40 acres with the maximum depth of 8 feet at each outlet structure and a mean

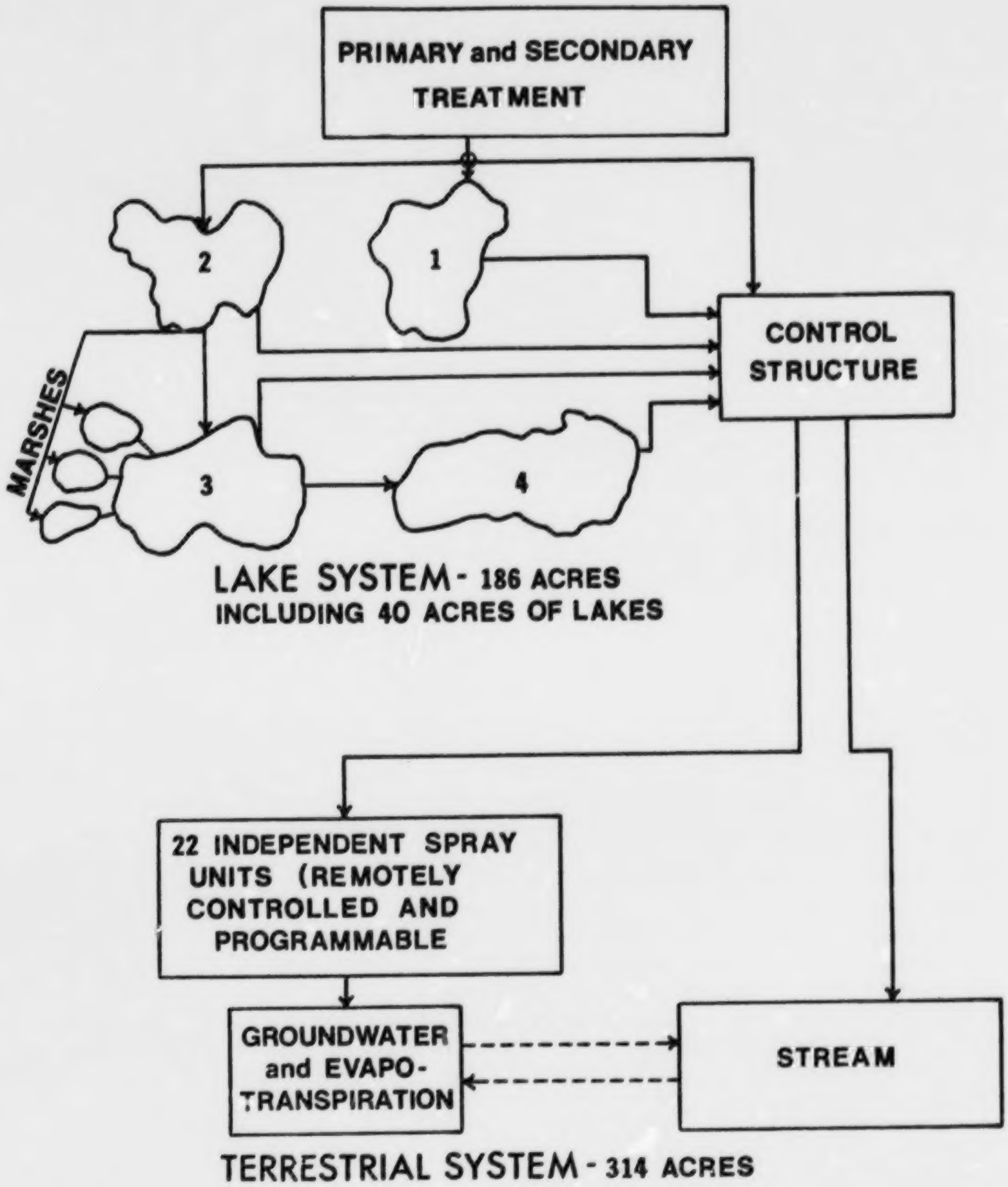
PRIMARY and SECONDARY TREATMENT
2
1
CONTROL STRUCTURE
MARSHES
3
4
LAKE SYSTEM - 186 ACRES INCLUDING 40 ACRES OF LAKES
22 INDEPENDENT SPRAY UNITS (REMOTELY CONTROLLED AND PROGRAMMABLE
GROUNDWATER and EVAPO-TRANSPIRATION
STREAM
TERRESTRIAL SYSTEM - 314 ACRES

Figure 2. Schematic of the Michigan State University wastewater recycling system.

depth of 6 feet. This depth was chosen to maintain the entire bottom within the euphotic zone in order to encourage the growth of rooted aquatic plants. The bottom was also contoured in a very uniform manner to ease their mechanical harvesting. Each lake has a collection basin at the outlet so that water may be lowered to collect fish or other aquatic fauna in a very small area. This area is serviced by a ramp to allow access by both boats and trucks. Control of discharge and lake level is afforded by both sliding gate valves and slash boards. The interlake transfer system and connection to the irrigation site is so designed that effluent can be taken directly from the pipeline or water can be intercepted at the discharge of any of the lakes in the system or mixed from any combination of lakes (see Figure 2). This feature will afford researchers with a wide range of water qualities to be applied and tested on the irrigation site.

MARSH SYSTEM

Marsh systems with their high rates of internal nutrient cycling may prove to be an extremely efficient system for the uptake and conversion of wastewater nutrients into useable products. To test the possibilities and feasibility of this idea we constructed three, one acre marshes which are fed from the discharge of Lake 2 in the system. Return water from the marshes enters Lake 3. The basins of the marshes were constructed in a terrace design that resulted in three zones of depths of 18, 24 and 36 inches. It is estimated that this will allow us to create in these basins biota quite comparable with natural marshes of this area.

In the construction of the lake basins and marshes particular attention was given to the sealing of the basins to prevent loss of water through the bottom soils. They were sealed with native clay and percolation tests indicated a low permiability of 0.07 inches per day.

LAND IRRIGATION SYSTEM

The last element of the system is a terrestrial site located on 350 acres immediately south of the lake system. As with much of the soils of the glaciated parts of Michigan and the Great Lakes basin there is a large array of soil types ranging from heavy clay to light sandy soils. Within the terrestrial site are forested areas, a pine plantation, cultivated fields and fields that have allowed to regress into old-field plant associations. The land is gently rolling and the entire area is drained by the Fenton outlet which enters a tributary of the Red Cedar River which in turn flows into the Grand River, a tributary of Lake Michigan (Figure 1). The irrigation site is bounded on three sides by an 800 foot buffer zone where we will not spray effluent at the present time but it is available for overland flow irrigation and research not requiring spraying of effluent.

Within the terrestrial area is a 145 acre spray irrigation site. Twenty-two individual valves, remotely controlled and programmable from the control building, direct the flow of water to the sets of surface irrigation pipe. Four, 700 foot tapered aluminum laterals extending north or south from a valve comprise a set. Water supply to each set is carried by a 21 inch underground pipe and the system is designed for winter operation. Spraying is by conventional Buckner 8600 sprinkler heads.

Within and surrounding both the lake and land sites we have drilled approximately 60 wells to monitor the level and quality of subsurface water. Depths vary from just a few feet into the glacial drift to over 200 feet, within the underlying sandstone aquifer. Approximately 1/3 of these wells will be equipped with automatic monitoring and sampling equipment as part of a major effort to characterize the subsurface hydrology in the region. All wells are cased, sealed and fully protected from external contamination. Surface water flow for the entire watershed in study will be measured by means of a network of weirs. Many of these are now installed. Climatological stations at selected points in the area provide the additional information to generate a detailed look at the entire hydrological cycle for the area.

TOTAL SYSTEM FLEXIBILITY

The physical facility as it was designed affords maximum flexibility in the development of research involving an integrated land and lake management system for the restoration of water quality and the recycling of nutrients. We have the potential for hydraulically stressing any area of the terrestrial system with effluent far in excess of its capacity of handling it to the extreme of no water application above that of natural rainfall. Within this range we have the capability of spraying at a variety of qualities as selected by the investigator, and on any temporal pattern that lends itself to research in the development of a particular management strategy. Table 1 gives the estimated loading rates of various chemical parameters if secondary effluent were directly applied to the irrigation site.

Table 1. Estimated soil loadings per acre on application of one inch of East Lansing Sewage Treatment Plant secondary effluent.

Chemical Parameter	Concentration in Secondary Effluent (mg/1)	Grams per Acre inch	Pounds per Acre Inch	Pounds per Acre Per Year **
Organic Nitrogen	2.2	227	0.5	36
Nitrate Nitrogen	3.07	317	0.7	50
Nitrite Nitrogen	0.25	26	0.06	4.1
Ammonia Nitrogen	9.70	1261	2.77	200
Soluble Phosphorus	1.1	143	0.3	23
Total Phosphorus	4.9	637	1.4	101
Total Carbon	150	15,450	34	2,445
Total Organic Carbon	30	3,090	6.8	489
Dissolved Organic Carbon	20	2,060	4.5	326
Suspended Solids	63	6,489	14.3	1,027
Volatile Solids	25	2,575	5.7	408
Chlorides	261	26,883	59.1	4,254
Iron*	0.81	83	0.18	13.2
Manganese	0.09	9	0.02	1.5
Zinc	0.19	20	0.04	3.1
Nickel	0.11	11	0.025	1.8
Copper	0.06	6	0.013	1.0
Mercury	0.00005	0.005	0.00001	0.0008

*Iron is being added for chemical phosphorus removal at the East Lansing Sewage Treatment Plant.

**At a rate of two inches per week between March and November (36 weeks).

Within the capabilities of the system we can chlorinate the effluent at several points including the point at which it leaves the sewage treatment plant, at the point where it enters the first of the lake series and at the point where it enters the pump and distribution center leading to the spray irrigation site. We can also chlorinate it before entrance into any natural surface outlet stream.

RESEARCH PLAN

AQUATIC STUDIES

The lakes will serve a multiplicity of purposes chief among which is the removal of nitrogen, phosphorus and other trace constituents of the wastewater. The mechanism planned to accomplish this is to incorporate these materials into the biological systems of the lake. The attenuation of influent material will come about by several mechanisms: (1) direct sedimentation to the lake bottoms; (2) incorporation into the plankton populations followed by precipitation or coprecipitation with the algae; (3) secondary incorporation into the animal populations of the lake including crayfish, tadpoles, minnows, aquatic insects, and food or forage fish; (4) aquatic macrophytes. These higher plants will constitute a major mechanism for concentrating and removal of dissolved materials from inflowing effluent.

During the fall of 1973 ten species of aquatic macrophytes were introduced into the lake system. Freshly cut material was transported to the campus lakes from nearby natural areas. Potamogeton foliosus was given general distribution over the entire extent of the lakes and localized clones of the following macrophytes were also established: P. pectinatus, P. crispus, Elodea canadensis, E. Nuttallii, Myriophyllum spicatum, Najas flexilis, Ranunculus sp. and Vallisneria americana. Care was taken to exclude problem species such as Ceratophyllum demersum. Based on the preliminary studies of McNabb and Tierney[3] we can expect net yields approximating 2100 gm/m^2 ash-free dry weight over a six month growing period. Of this weight about 1.5% is P and 5% is N.

As the water flows from lake 1 through the sequence to lake 2, it can be diverted to 3 one acre marshes adjoining lake 2. The purpose of the marshes is to test the efficacy of a marsh community in removing nutrients from wastewater. There is some evidence that they are unusually proficient in denitrification processes. The research strategy on these is to plant and develop marsh communities adaptable to the uptake of high levels of nutrients, then pass effluent through these three marshes at a rate compatable with the maximum uptake of nutrient materials. The control of the marshes is individual and the flow can be regulated at different levels. Cognate research on the utilization of these marshes by waterfowl and wildlife will be undertaken in detail. It is anticipated that they will produce a harvestable crop of wildlife as well as serve for basic research.

TERRESTRIAL STUDIES

Forest

Within the terrestrial system is an area supporting a mixed hardwood forest typical of most woodlots in southern Michigan. Here, the survival and growth of the trees will be documented under different spray application regimes and the penetration, uptake of nutrients, and loss from the system will be measured by a variety of techniques. Partially covered by the spray irrigation system is a pine plantation which will serve as contrast for the deciduous forest system. Plans for additional research include the planting of trees that can be used for intensive cropping on a short term rotation. It is presumed that these will be used for pulp wood and their complete removal and utilization will serve to take the accumulated nitrogen and phosphorus that they have incorporated during their growth completely out of the system.

Incorporated as a by-product of the forest research will be a study not only of the nutrients removed from the system but also a study of the return and internal cycling of these materials in the form of leaf fall to the forest soils. This offers the possibility of the selection and management of tree types for maximum binding and holding of those nutrients that we desire to either remove from the system or have tied up in such a manner that they will not enter the potable water aquifer.

Old Field - Plant Ecosystems

Preliminary work over the past several years has indicated that the natural diversity of an old-field community may be one of the most effective and efficient units for capturing nutrients from the wastewaters sprayed on the land. There is also evidence from these studies that spraying them with nutrient rich effluent will appreciably change the composition of the plant community. The efficiency of these plant communities in trapping nutrients and the role of grazers, especially insects, in transporting nutrient material out of the study system will be a topic of continuing research.

Cultivated Crops

In the demonstration phase of the Michigan State University program we are not only striving to clean up the wastewaters of a city, but to also make use of those materials in the wastewater that have been mined and fabricated at considerable costs of natural resources and energy. It is self evident that the transformation of these resources back into useable food or fiber is an essential ingredient in this demonstration. Thus, we will be carrying out in-depth research on those crops that can be used in this way.

Many crops now cultivated for either direct or indirect human use can be benefited by the irrigation with nutrient rich waters. But in addition to testing the efficiency of these known plant crops we will undertake a wide spread search for plants that meet the rather unique conditions that we have set up in our recycling program. Over the centuries crops have been selected for many characteristics among which are the ability to live with a minimum of water and grow with a minimum of input of nutrients. There is now a need to reverse this particular selection and choose plants that will thrive in an excess of water and in rich concentrations of those nutrients that promote growth. With these crops, when they are found or developed from the gene pool of known and possible crops of the world, we will again have to select for palitability and other factors desirable in plants to be used for direct or indirect food production.

It is recognized that certain plants take up heavy metals and other materials that may be found in wastewater effluents and that these levels may reach the point where they are unacceptable for either human or domestic animal food. Before such judgements can be made it will be necessary to demonstrate such uptake and consider the possibilities of either rejecting these forms as food or seeking new uses for them. Since one of the goals of such a project is to keep heavy metals and other potentially hazardous materials out of our streams and lakes it appears desirable to concentrate them in plants produced in aquatic or terrestrial systems. The problem of their utilization would then become the focus of additional research. In this manner we at least have the hazardous materials "in hand."

Soil Research

Any system such as the one proposed at Michigan State University will be dependent to an important degree on the: (1) selection of a site with soils compatable with the concept of total waste management and recycling; and (2) management of the soil complex in such a manner as to accomplish the mission of the project over a long period (approaching steady state); and (3) a subsurface geology that both serves as a filter and does not present a barrier to water movement downward from the water application site.

Detailed studies will be carried out on both the short and long range effects on soil texture and composition of heavy applicatons of wastewater. This will involve studies of the retention of heavy metals in the soil mantel and development of strategies that will allow the heavy application of nitrogen in its several forms and yet allow the transformation and uptake of the nitrogen into forms that will either be held in the crops or be driven off to the atmosphere in the form of gaseous nitrogen.

The site selected has a great variety of soils characteristic of the Great Lakes drainage basin thus affording the opportunity for in-depth research on wastewater application to diverse soil types.

MONITORING PLAN

CHEMICAL AND PHYSICAL

As both a service and as a basic research effort there is a need for continuous monitoring of a large array of chemical and physical parameters connected with both the operation of the wastewater treatment plant and with the lake and land facets of the recycling system. As a service to all investigators working on the total project, there will be available data on 52 chemical parameters representing collections starting with the raw sewage entering the sewage treatment plant, the attenuation and removal of materials as they move through primary and secondary treatment and as they enter the pipeline leading to the lake system. The chemical parameters of interest will be checked as they move through the lake system from one lake to the next and into the pump station. Similar and further tests will be made through the stream outfall system and to the several areas of the spray irrigation site. A similar series of analyses will be made from the test well system. These will identify movement of chemical materials into the soil mantel and down into the subsoil and ground water aquifer if such movement does take place.

Most chemical parameters will be serviced by the Institute of Water Research's chemical laboratory under the direction of an analytical chemist and his staff. Within the limitations of modern technology we will monitor the chemical parameters through automated equipment both for the savings in time and cost and for uniformity of results. There will be a constant check on the quality of the chemical work through the splitting of samples to outside laboratories and duplication of analyses within the laboratory. This program will also be closely coordinated with standardization methodologies set forth in the International Joint Commission's program on pollution of the Great Lakes.

MICROBIOLOGICAL

One of the most widespread of all criticism of recycling of wastewater has been in the arena of public health concern. There appears to be general acceptance that the bacterial content of such water can be controlled and reduced to completely acceptable levels through chlorination at some stage in the recycling process. However, there have been expressed doubts about the safety of using recycled sewage effluent, regardless of the chemical and biological stages that it has passed through. We know that such recharges into the ground aquifers are widespread in areas where sewage disposal plants empty their wastes into streams which in turn recharge ground aquifers. However, in designing a recycling system it is essential that all concerns of this type be answered fully and that the final product of such a system be completely safe from a health standpoint.

One of the major problems in identification of the health hazards from a recycling system has been the high cost and inadequate technology in identifying many of the more important viruses in dilute solutions such as one would find in the recharge to a ground water aquifer. To address ourselves to this problem we are establishing a fully equipped laboratory capable of both routine and research analytical efforts in determination of the total microbiological problems involved in this project. Paralleling the chemical monitoring program, the microbiological samples for both bacteria and virus will be taken at every major transfer point within the system. This will include identification from the raw sewage through the treatment plant, through the pipeline to the lake system and to all aspects of the spray irrigation site. This will also include a detailed consideration of the potential for dispersent by aerosols in the spray irrigation process.

SUMMARY

The total recycling concept that we are preparing to test is based upon:

(1) The fact that our natural resources are limited in supply and will, within the foreseeable future, become critical both to industry and agriculture. This is particularly true of phosphorus and is well demonstrated by the fact that the world is now competing for a relatively limited supply of available phosphorus to feed a vastly increasing world population.

(2) That materials, such as nitrogen, found in abundance in our wastewaters will be in short supply immediately and are of concern as well because of the high levels of energy that they require to manufacture them.

(3) These two essential nutrients are very great contributors to the pollution of our streams, our inland lakes, and the Great Lakes.

Thus, we believe that for the conservation of our essential natural resources and for the reduction in the use of energy and the protection of our water resources of the country, it is essential that we both remove these nutrients from the waters and also make use of them in the production of food materials. The mechanism for doing this is in part understood and in part there is a serious lack of scientific knowledge upon which to base the recycling and reuse of materials contained in wastewater.

Recently, in an unusual joint release, the Water Pollution Control Federation and the American Water Works Association stated their support for a massive research effort to develop needed technology and evaluate the potential health problems related to recycling of wastewaters to domestic water supplies. Noting that "sound management of our total available water resources must include consideration of the potential use of properly treated wastewaters as part of drinking water supplies," the joint resolution pointed out the lack of adequate scientific information about possible acute and long term effects on man's health for such reuse, and also noted that the essential fail-safe technology to permit such direct reuse has not yet been demonstrated. The resolution emphasized the need for prompt action based on the fact that "ever greater amounts of treated wastewaters are being discharged to the waterways of the nation and constitute an increasing proportion of many existing water supplies." Although indirect recycling has occurred for many years with no obvious indications of damage to the public health, almost no effort has been devoted to the complex and long term evaluation and quantification of possible hazards related to the ingestion of recycled wastewater or its by-products. Furthermore, the nature of hazardous materials in wastewaters will require many years of research to develop monitoring and treatment processes essential to their removal or control.

Since this is a problem with applications throughout the entire United States, the federal government can play a major role in providing the kind of funds and coordination needed to implement the research, monitoring, and pilot programs that must be developed to forward the progress in this effort. Because of the interrelated nature of the many facets of a program such as this, it may well fall to those universities with a large and diverse staff to undertake an overall evaluation (systems analysis) of the process and to develop demonstration and educational programs that must accompany such an effort. This is what is proposed in the Michigan State University program.

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EXPERIENCES WITH A MARINE AQUACULTURE-TERTIARY SEWAGE TREATMENT COMPLEX

John E. Huguenin*

John H. Ryther

INTRODUCTION

Treated secondary sewage effluent has been shown to be a good complete fertilizer for the growth of microscopic marine plants (phytoplankton), which form the base of the marine food chain (Dunstan and Menzel, 1971[1]). From this somewhat surprising fact has emerged a concept for a combined aquaculture-tertiary sewage treatment system (Ryther, Dunstan, Tenore and Huguenin, 1972[2]), which has been under active investigation at the Woods Hole Oceanographic Institution since late 1969. While the optimal operating points for the two objectives of nutrient removal and maximizing aquacultural output are not coincident, they are close enough to make a combined system feasible.

The essence of the concept is a treated sewage-marine phytoplankton-bivalve mollusk (oysters, clams, mussels, scallops) food chain. However, in reality it includes a much more complex food web with current research involving the utilization of flounder, lobster, abalone, bait worms and seaweeds as secondary components in such a system. This is due not only to a desire to hedge against uncertainties, but is also based on experimental results which indicate that multi-species aquaculture systems are more efficient and productive than those with only one species (Tenore, Goldman, and Clarner, 1973[3]). Thus, efforts are leading to the development not of a single system but rather to a spectrum of technically feasible systems. Efforts are also underway to investigate the legal, political, regulatory, marketing and public health aspects of such a concept. In addition, once

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established, multi-species systems have value as research tools in their own right, since complex natural processes involving different trophic levels can be investigated under controlled conditions. While a lot of work remains to be done, from experience to date both indoors and outside (Dunstan and Tenore, 1972[4]; Tenore and Dunstan, 1973a[5]), it is clear that such systems can be made to efficiently remove nutrients (Goldman, Tenore, Ryther and Corwin, 1974[6]; Goldman, Tenore and Stanley, 1974[7]) and produce rapid growth of marine organisms with high survival.

Encouraging results and the need for better seawater research facilities to more realistically evaluate the concept for practical applications led to the design and construction of the Environmental Systems Laboratoty (ESL) situated in Woods Hole, Massachusetts. The primary intent was to provide a flexible experimental facility to enable research over the complete spectrum of possible development for such systems.

Unfortunately, the ultimate application of the concept in New England would require the use of waste heat from power plants to enable the animals to feed during the winter. Due to this complication, these same ideas may prove simpler to implement on a large scale in areas of the country with more seasonally uniform water temperatures. Small scale experimentation has started at a site in Florida to evaluate this concept in a semi-tropical environment, and a larger scale facility in that area is now in the planning stage. For the same reason, the U.S. Pacific Coast is also of interest.

DESIGN PROBLEMS

In terms of system design, the nature of the nutrient source (i.e., secondary sewage or not) is for most purposes irrelevant. The major design problems are those that would be common to any other research oriented marine aquaculture system of equal complexity. The main design constraint was clearly uncertainty over system requirements. This was due to both the continually changing research experiments and the evolution and refinement of the concept itself. Since quantitative requirements could only be defined with confidence for the relatively near term, at most about two years, designing for system flexibility and amenability to modifications became very important.

An interesting fact is that much of the necessary biological information needed for the development of aquaculture systems is not available. This problem exists for several reasons, even for species that have been relatively well studied. In spite of the tremendous amount of information available on the biology of some marine animals, the data from recognized experts can cover such a range of values as to be useless. As an example, the published respiration rate of oysters varied by a factor of more than twenty. On top of this uncertainty was the fact that within the components of our system there are many other sources and sinks for dissolved oxygen. Also surprisingly, important design variables have sometimes never been investigated and published in spite of the voluminous literature available. For us such a critical variable was the efficiency with which shellfish convert different kinds of food to shellfish meat (Tenore and Dunstan, 1973b[8], 1973c[9]).

Since the individual circumstances surrounding any particular operation can so easily alter the biology of the system, the best approach, even where apparently good information exists, is the actual testing of small scale systems under conditions as close as possible to those to be encountered. While such testing can be very useful in reducing the risks and developing nominal relationships and sizing criteria, there are nevertheless some remaining pitfalls, especially if indistinct research objectives are involved. Even for commercial operations whose design objectives can be quite unambiguous, there are problems of determining system economics and the optimum scale for operations as well as risks of major changes in system configuration dictated by improvements in state-of-the-art, market considerations, or institutional factors.

The engineering problems to be encountered include those due to the corrosive properties of seawater, toxicity of many materials to marine organisms, biological fouling of pipes and equipment, and the lack of any readily available sources of bioengineering design information. On any specific project, areas involving design risk should be recognized and acknowledged. Many of these risks, while not capable of being completely eliminated, can be reduced by collecting information on previous or related endeavors (Clark and Clark, 1964[10]; Lasker and Vlymen, 1969[11]; Pruder, Epifanio and Malout, 1973[12]; Strober, 1972[13]), by small scale testing, and by careful design to minimize the consequences of bad judgements. Due to the large uncertainties in aquaculture systems, there is a high premium on flexible designs and systems with the capability to readily undergo modifications to meet changing requirements.

DESIGN FEATURES

The basic configuration adopted for the facility designed, at least in part, as a "pilot plant" for the previously described concept is shown on Figure 1. This system, from two head boxes, is a gravity flow system with the six ponds, eight raceways and settling pond in order of decreasing elevation. The settling pond and algae ponds are prepared sand surfaces covered with 20 mil plastic liners, a relatively inexpensive form of construction readily expandable to much larger sizes. The raceways are concrete. It will be noted that experiments can be conducted in an indoor wet lab, on a very flexible outdoor test area and in the larger units, providing a choice of experimental sizes. Figure 2 shows the present piping configuration of

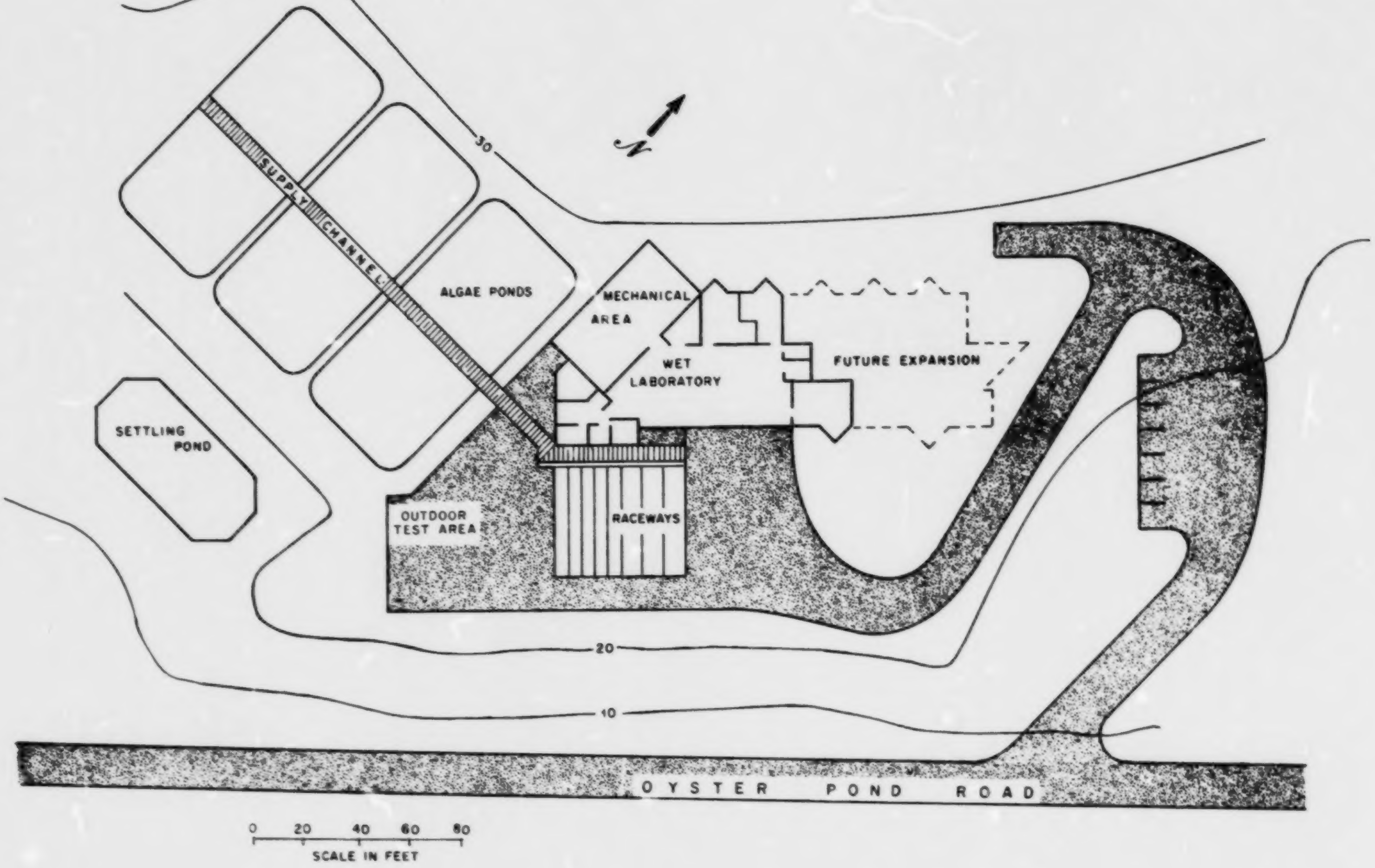
SUPPLY CHANNEL
ALGAE PONDS
30
N
MECHANICAL AREA
WET LABORATORY
FUTURE EXPANSION
SETTLING POND
OUTDOOR TEST AREA
RACEWAYS
20
10
OYSTER POND ROAD
0 20 40 60 80
SCALE IN FEET

Figure 1 ENVIRONMENTAL SYSTEMS LABORATORY
WOODS HOLE OCEANOGRAPHIC INSTITUTION

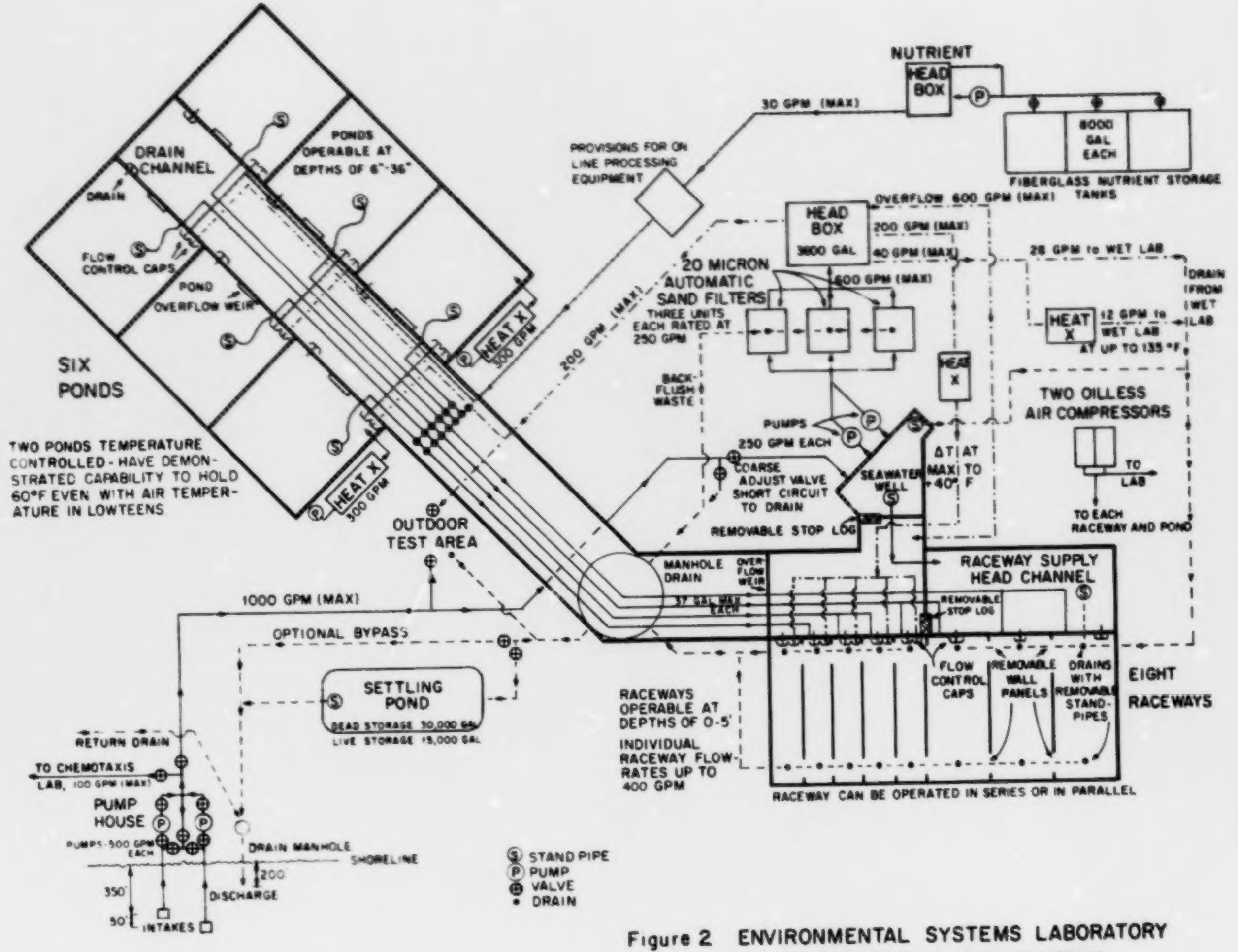
NUTRIENT
HEAD BOX
30 GPM (MAX)
8000 GAL EACH
FIBERGLASS NUTRIENT STORAGE TANKS
PROVISIONS FOR ON LINE PROCESSING EQUIPMENT
OVERFLOW 600 GPM (MAX)
HEAD BOX 3600 GAL
200 GPM (MAX)
40 GPM (MAX)
600 GPM (MAX)
20 MICRON AUTOMATIC SAND FILTERS
THREE UNITS EACH RATED AT 250 GPM
BACK-FLUSH WASTE
28 GPM to WET LAB
DRAIN FROM WET LAB
HEAT X
12 GPM to WET LAB AT UP TO 135 °F
HEAT X
TWO OILLESS AIR COMPRESSORS
TO LAB
TO EACH RACEWAY AND POND
PUMPS 250 GPM EACH
ΔT AT MAX TO 40° F
COARSE ADJUST VALVE SHORT CIRCUIT TO DRAIN
SEAWATER WELL
REMOVABLE STOP LOG
DRAIN CHANNEL
DRAIN
PONDS OPERABLE AT DEPTHS OF 6"-36"
FLOW CONTROL CAPS
POND OVERFLOW WEIR
SIX PONDS
HEAT X 300 GPM
HEAT X 300 GPM
200 GPM (MAX)
TWO PONDS TEMPERATURE CONTROLLED - HAVE DEMONSTRATED CAPABILITY TO HOLD 60°F EVEN WITH AIR TEMPERATURE IN LOWTEENS
OUTDOOR TEST AREA
MANHOLE DRAIN
OVERFLOW WEIR
37 GAL MAX EACH
RACEWAY SUPPLY HEAD CHANNEL
REMOVABLE STOP LOG
FLOW CONTROL CAPS
REMOVABLE WALL PANELS
DRAINS WITH REMOVABLE STANDPIPES
EIGHT RACEWAYS
1000 GPM (MAX)
OPTIONAL BYPASS
SETTLING POND
DEAD STORAGE 30,000 GAL
LIVE STORAGE 15,000 GAL
RACEWAYS OPERABLE AT DEPTHS OF 0-5'
INDIVIDUAL RACEWAY FLOWRATES UP TO 400 GPM
RACEWAY CAN BE OPERATED IN SERIES OR IN PARALLEL
RETURN DRAIN
TO CHEMOTAXIS LAB, 100 GPM (MAX)
PUMP HOUSE
PUMPS-500 GPM EACH
DRAIN MANHOLE
SHORELINE
350'
50'
200
INTAKES
DISCHARGE
S STAND PIPE
P PUMP
⊕ VALVE
• DRAIN

Figure 2 ENVIRONMENTAL SYSTEMS LABORATORY SCHEMATIC FLOW DIAGRAM

the physical plant. This figure indicates that the outdoor units can be operated with a variety of water depths and flow conditions as well as enabling sequential on line operations in physically separated units. Services such as raw seawater, filtered seawater, nutrient supply, temperature controlled seawater, fresh water, electric power, drains and oilless compressed air are available in many locations and could easily be provided at others. The pipe materials are high density polyethylene for the main seawater lines to and from the beach and polyvinyl chloride (PVC) for most of the smaller on-site piping. Care was taken in the design to assure easy access, for maintenance and modification, to as much of this piping as was possible.

Several of the subsystems give the facility considerable added research capability. A separate nutrient distribution system, which includes three 8,000 gal. fiberglass tanks, a headbox and piping, can distribute any pumpable fluid, such as secondary sewage, an artificial media, a food slurry or even a "pullutant", to individual ponds or raceways. The four impervious-carbon heat exchangers provide a significant controllable seawater heating capability. This capability can remove the effects of seasonal water temperature variants as an uncontrolled variable in experiments, enables year round operations and enables reasonably large-scale research on thermal effects to be performed.

The sizes of the larger outdoor units were chosen to provide results and experiences applicable to much larger facilities and still have an acceptable initial capital cost. Due to the multi-objective character of this facility the relative number and sizes of the components are not necessarily those indicated by scaling up the sewage-algae-shellfish system. If other research objectives were to be sacrificed, this complex could ultimately treat the effluents (if available) of up to 500 people and perhaps produce around four tons of shellfish meat annually. However, it must be remembered that the primary output of this facility is technical information and that the economics of a research group and its facilities are vastly different from those necessary for commercial operations. A facility built for commercial use would have been designed much differently.

EXPERIENCES TO DATE

The major construction activities were completed in the fall of 1973 and the seawater system was permanently activated on October 30. The next day 300,000 juvenile oysters arrived and initial operation commenced.

The pond design has so far proven very satisfactory. In spite of a good deal of activity there have been no serious problems with punctures of the polyvinyl chloride liners. A cinder block dropped on its corner produced only a small puncture in one of the ponds, which was easily patched. Some of the ponds have been frozen so solidly that people could walk on the ice and no damage to the liners has been observed. In addition, the liners can be readily cleaned, even after being heavily fouled with filamentous algae. Still to be determined is the long term performance of the liners, especially with respect to sun damage. The expected useful life is five years but other, slightly more expensive, liner materials are stated to not be subject to this damage.

Overall, winter operations of the total complex have given problems far less severe than anticipated. Performance of the heating and circulatory system provided for two of the six ponds has been better than planned. We have been able to maintain water temperatures in these as high as 75°F, but more normally set at about 60°F, even with air temperatures in the low teens and with lower than anticipated heat additions. Dense continuous flow marine phytoplankton cultures of up to 2×10^6 diatoms/ml have been easily maintained for many weeks in these ponds with a daily harvest of 30% of the culture volume. The densities of these cultures are considerably higher than those achieved in our smaller scale experiments. We believe these 36,000 gallon diatom cultures to be the largest in the world. Unfortunately, due to the lack of a large tank truck, these large cultures have so far been operated with concentrated liquid fertilizers rather than with secondary sewage as originally intended.

The phytoplankton grown this winter have been fed continuously on an in-line basis to 300,000 juvenile oysters and more recently 150,000 juvenile hard clams (quahogs) maintained in raceways supplied with heated (60°F) filtered (20 micron) seawater. The temperature drop across a forty foot concrete raceway, even under very cold conditions and low flow through times (about 8 hours), has been less than 2°F. It is too early to say anything about the winter growth of these shellfish, except that it has been substantial.

Several aspects of our operations, however, have proven troublesome. The five specialized carbon lined pumps with carbon impellers have provided many problems, mostly in the area of not meeting performance specifications and excessive water leakage at seals. In addition, a planned traveling service platform has not yet been procured, making access and the handling of stacked shellfish trays and other loads in the raceways very difficult. This unit and a large tank truck are currently the only important components still missing from the system.

More biologically oriented problems have also arisen. The valance heaters installed to heat the building were manufactured with a coating of some hydrocarbon, which has contaminated the building but is gradually burning off and, fortunately, does not seem to have affected our current research. Undesirable growths on the sides and bottoms of the ponds and raceways have been less of a problem than anticipated, but are expected to get worse with the coming of spring and summer. As the cultures in the algae ponds get denser the undesirable growth on the sides is markedly reduced. This is a result which was not predicted from our smaller scale experiments. However, removing fouling and accumulated organic debris is still a large potential sink for man-hours and the development of satisfactory methods and equipment to minimize the problem may be one of the keys to practical forms of marine aquaculture.

OUTLOOK FOR THE FUTURE

Several potential problems must be investigated and resolved before the promising work already done on the use of treated sewage in marine aquaculture can be beneficially applied on a large scale. All potential public health hazards (Shuval and Gruener, 1973[14]) must be thoroughly investigated and where real hazards are found to exist, means must be found to eliminate or circumvent the problem. The threat from enteric virus and the possible presence of some carcinogenic chemicals are two areas of particular importance. If such research is not done there is little likelihood of acceptance of this type of system by either the public or the regulatory agencies. In addition, continual small scale controlled experimentation must be carried out with the purpose of increasing efficiency and learning more about system dynamics. The gains from such experimentation can be substantial. As an example, during the year and a half between the preliminary design phase and the construction of the ESL, the treated sewage handling capacity of the ponds (size being constant) increased by more than 300% and the required seawater flow to the raceways was cut 90%. Additional improvements are a distinct possibility.

The current ESL is a subscale pilot plant with multiple objectives. No attempt was made in the design to enable its potential "revenue" to exceed its costs, since its small physical size, the size and composition of its staff, and its flexible research objectives precluded this possibility. The facility will, however, produce adequate quantities of waste-grown seafoods for testing in related research projects and possibly even for the critical function of preliminary market testing. The emphasis has not been on maximizing production but rather on conclusively proving technical feasibility at a realistic scale of operation and to exploring the practicality of such systems for large scale applications. While technical feasibility appears relatively assured, the economics and acceptability of the ultimate system cannot yet be determined. They are contingent on a great many factors including methods and equipment still to be developed.

A few years of experience with the ESL in improving system efficiency, researching risk areas, developing better monitoring and control methods, and reducing production costs is needed to provide the information necessary for realistic evaluation and decision making regarding the value of the basic concept. With this and similar projects providing the needed design information, full scale demonstration projects may shortly become realistic possibilities.

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POLYCULTURAL WASTEWATER RECLAMATION AT CALIFORNIA POLYTECHNIC STATE UNIVERSITY - AN ACADEMIC INSTRUCTIONAL SYSTEM

by

Dr. Richard J. Krejsa *

INTRODUCTION

When I attended Michigan State College in the early 50's, it was called a "cow college" partly because of the taunting disdain of midwestern football rivals, and partly out of respect for the college's self-reliance on campus grown food and dairy products. I am now on the faculty of California Polytechnic State University, which also once was a self-sufficient "cow college". While it is not feasible to return to complete self-sufficiency, we are actively exploring ways of regaining a certain amount of integrated independence wherever possible. This presentation constitutes a progress report of one such attempt.

Our polycultural wastewater reclamation system is still more a concept than an accomplished reality. It is part of an educational experiment based on the realization that a multi-disciplinary approach to societal and technological problems is necessary.

Goal

Our goal, basically is to make a resource out of wastewater, and to educate students and the public in the methods and philosophy necessary to do so.

Objectives

The objectives of our proposed polycultural wastewater reclamation system are four fold:

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** Portions of this investigation were supported by a grant from the Creative Activity Research Effort (CARE) program at Cal Poly, and by funds allocated to student projects through the Student Enterprise Program of the Cal Poly Foundation.

1) To propose and investigate alternative biological and agricultural strategies of secondary treated wastewater reclamation and reutilization;
2) To monitor changes in water and soil quality attendant with the processing of wastewater through our polyculture system;
3) To introduce students to practical and innovative technologies;
4) To educate the general public that "waste" water need not be wasted.

Accomplishments To Date

Over a two year period, six successive classes of Agricultural Engineering students built the 50 acre-foot reservoir (Fig.1) as a practical class project while learning how to operate tractors, dozers and other heavy equipment. This reservoir will serve as the Biological Treatment Lagoon. Other Ag Engineering students designed and constructed experimental closed-system aquaculture units for rearing catfish and trout. Still others constructed automatic feeders, and bulk harvesting equipment, and compiled specific bibliographies on the engineering aspects of aquaculture.

A student in Animal Science grew St. Peter's fish, Tilapia mossambica, brown bullheads, Ictalurus nebulosus and clams, Corbicula manillensis(?), in an experimental backyard polyculture greenhouse[1]. A Crops Science student tested the utility of secondary effluent on certain grass crops. Students in an Ag Business Management class in communications surveyed local consumer attitudes on the acceptance of farm grown catfish and successfully promoted the sale of farm grown fish at a local chain supermarket.

A Biological Science student compared growth patterns of cage-cultured channel catfish reared in different population densities (Fig.2a,b)[2]. Another biology student (Fig.2c) concocted an experimental fish food made from dehydrated steer offal, cafeteria kitchen scraps, fish meal, and agricultural crop wastes, and then pelletized in our campus feed mill. The growth rates of cage-cultured catfish fed on the experimental diet were compared to those from control fish fed on standard Trout Chow.

Another young biologist studied the behavior of hatching catfish fry and helped design new incubation and hatching techniques. Others yet compared the behavior and growth of fingerling catfish in normal irrigation water and the secondary effluent from the California Men's Colony treatment plant, studied the ultrastructure of developing cat-

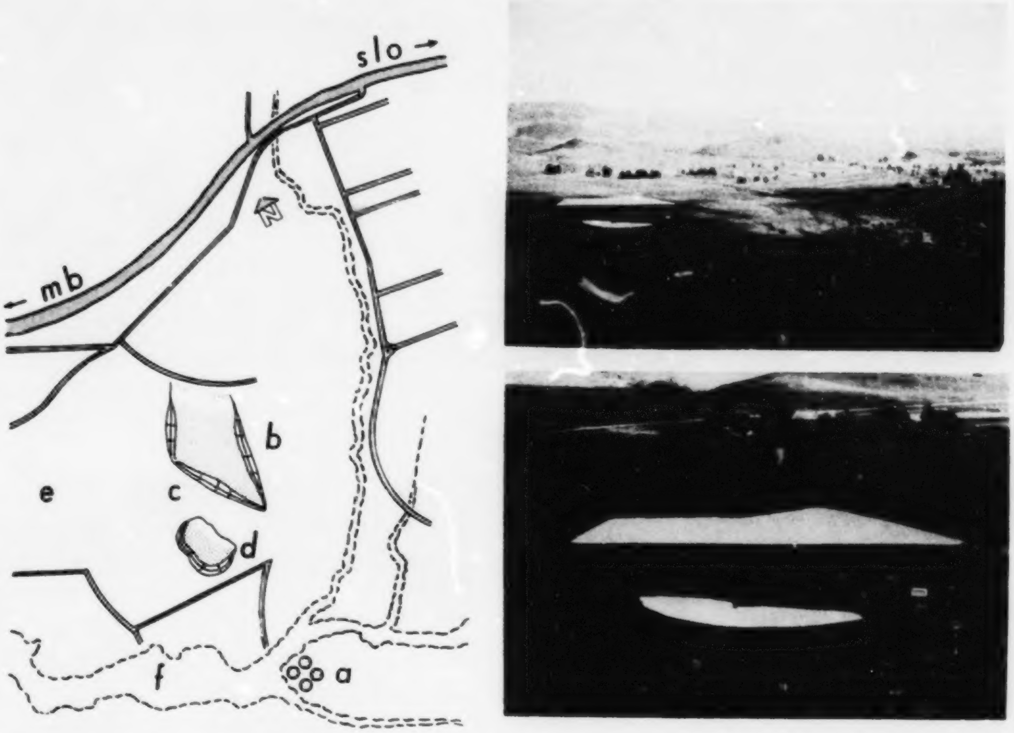
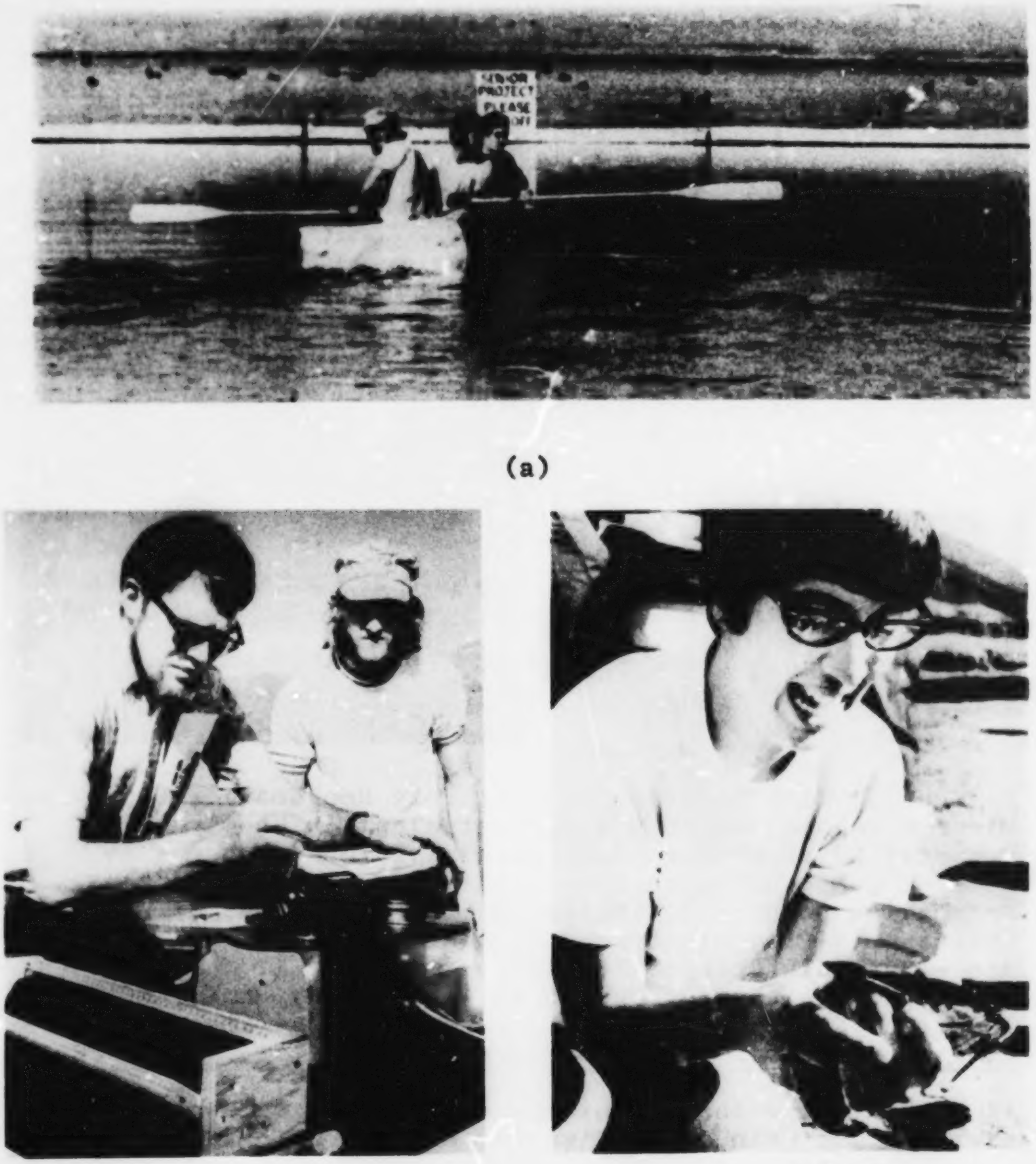


Figure 1. Site Location of Cal Poly Reclamation Unit. Diagram legend - a. CMC treatment plant; b. newly constructed 50 acre-foot Biological Treatment Lagoon; c. site of proposed aquaculture units; d. 10 acre-foot irrigation ponds; e. irrigated crop land area; f. Chorro Creek; mb = City of Morro Bay, 5 miles west on Highway 1; slo = San Luis Obispo, 7 miles east.
Upper photo - view of Chorro Valley looking east, CMC Treatment Plant on the right of reservoirs. Lower photo - view of reservoir area, looking east.

fish skin using the techniques of electron microscopy, and examined the skin of catfish for evidence of bacteriological pathogens.

Two students from the School of Architecture and Environmental Design did their senior project on the design of a simple, efficient experimental fish hatchery using space frame technology[3]. A class of 12 design students spent a portion of the academic year running structural analyses of the roof design created by the two senior project students. Still another design class of 32 students spent



(a)

(b) (c)

Figure 2. Aquacultural experimentation at Cal Poly. a. Floating Cage culture density/growth experiment area. Sheppard Lake; b. Weighing channel catfish grown in cage culture; c. Sampling channel catfish grown on experimental diet concocted from wastes.

the recent winter quarter attempting to incorporate and synthesize the multi-purpose aspects of the proposed polyculture reclamation project into a functional whole. They came up with several innovative design concepts, two of which are presented here (Figs. 3,4).

All of these preliminary and exploratory analyses were done with minimum expense and maximum enthusiasm. The construction of the new 50-acre foot reservoir was completed last Spring and it was filled for the first time last Summer.

The program announces this meeting as "The First Interdisplinary Conference of Its Kind". As can be seen above, our Cal Poly project has been interdisciplinary from its inception over two years ago. Even if the production aspects of the project were to go no further now, we would deem the educational value of the project as worthwhile and valid.

Historical and Political Perspective

The current form of the Cal Poly project evolved as the result of the interplay of several factors, some of which have been described above, and others which will be described from a historical and political perspective below.

Consumptive water uses within the County of San Luis Obispo, as well as throughout the Central Coast region are taxing the capability of the watersheds and groundwater basins. With certain exceptions, local public policy up to the early 70's has not faced the water problem squarely. Projected growth in population and irrigation development, anticipated for the late 60's did not fully occur in all areas; however in areas of the County where it occurred and carried over into the 70's, overdrafting and mining of the local groundwater supply has resulted. To citizens of these areas, such activities provide a false sense of security regarding the permanence and reliability of their groundwater resources. As long as water can be imported from elsewhere, water conservation and quality objectives are overlooked. There is a need for consumer education.

The problem of water conservation and quality is not limited to the Central Coast region[4]. Indeed for years California water resources have been managed for their utility, expecially for consumptive uses. In 1968-69, for example, 76 water treatment facilities, each with design capacities greater than one million gallons daily (m.g.d.) serviced 90% of the population of 8 coastal Southern California counties. Yet only 3.8% (45,000 acre-feet) of

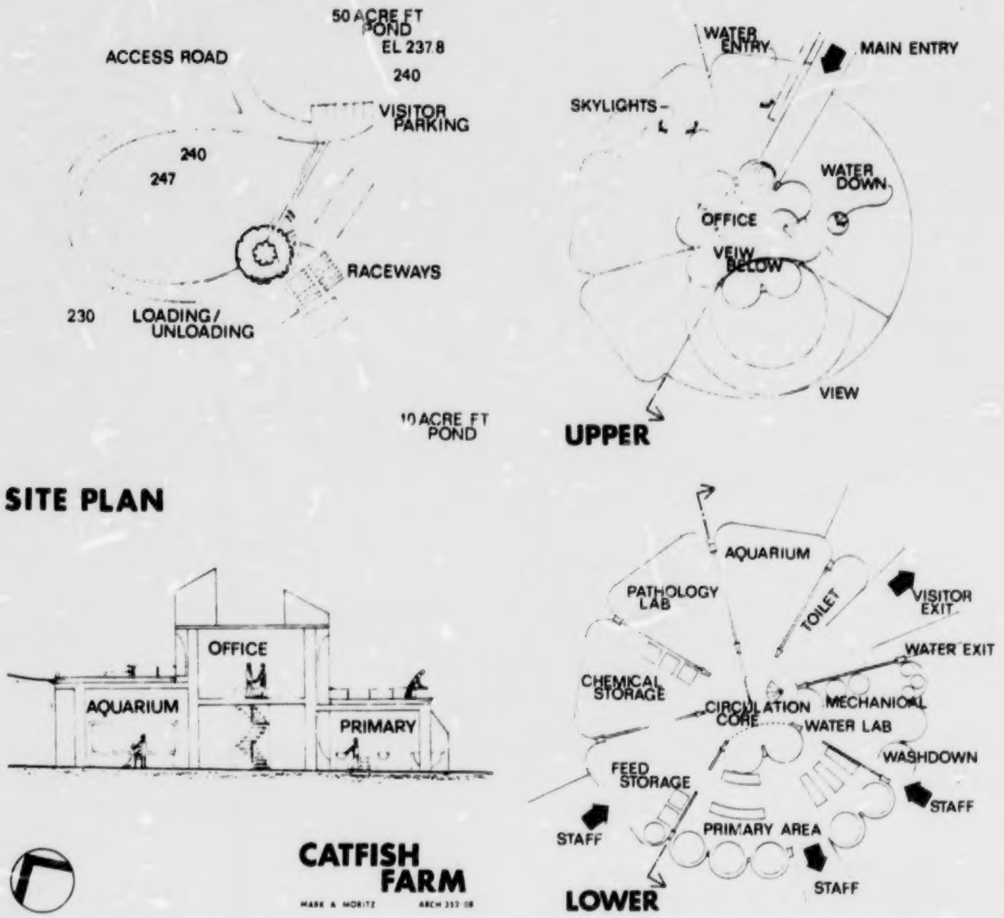

50 ACRE FT POND
EL 237.8
240
ACCESS ROAD
VISITOR PARKING
240
247
RACEWAYS
230
LOADING/ UNLOADING
10 ACRE FT POND
SITE PLAN
WATER ENTRY
MAIN ENTRY
SKYLIGHTS
WATER DOWN
OFFICE
VEIW BELOW
VIEW
UPPER
AQUARIUM
PATHOLOGY LAB
TOILET
VISITOR EXIT
WATER EXIT
CHEMICAL STORAGE
CIRCULATION CORE
MECHANICAL
WATER LAB
WASHDOWN
FEED STORAGE
STAFF
PRIMARY AREA
STAFF
STAFF
LOWER
OFFICE
AQUARIUM
PRIMARY
CATFISH FARM
MARK A MORITZ

Figure 3. Concept of a polycultural wastewater reclamation plant designed by architecture student Mark Moritz. The basic symbolic design innovation here is that the unit is made out of repeating 8 foot diameter sections of concrete sewer pipe. As visitors enter the parking area, they pass under a large sewer pipe transporting treated water from the 50 acre-foot reservoir. They then approach the hatchery/lab via a rampway paralleling the pipe. As water from the pipe discharges onto the tiered concrete roof, it is heated by the sun, ultimately flowing down for use in the lab (water down), and out for use in the raceway and pond area. Visitors entering on the upper level descend into the lower level where they can view the primary area without interfering with lab procedures.

the total wastewater discharged from these plants were reused for irrigation of crops and/or recreational parks, or for planned groundwater recharge. The remainder was used only once before its discharge directly into the Pacific Ocean (84%) or variously disposed onto land or into fresh water streams (12.2%)[5].

As recently as June, 1971. the same memorandum report of the California Department of Water Resources stated that, for the immediate future, the areas of San Luis Obispo County served by four of the five large capacity coastal wastewater plants had an abundant supply of local, low-cost water of good quality, and therefore "increased use of treated wastewater....is not probable"[5].

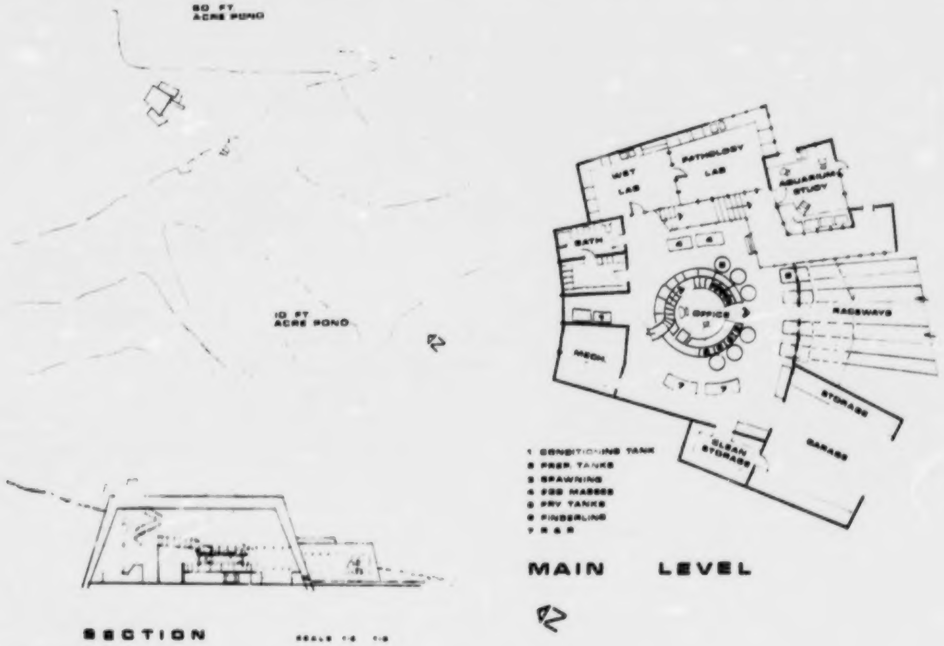

80 FT ACRE POND
10 FT ACRE POND
WET LAB
PATHOLOGY LAB
AQUARIUM STUDY
BATH
OFFICE
MECH
RACEWAYS
STORAGE
CLEAN STORAGE
GARAGE
1 CONDITIONING TANK
2 PREP. TANKS
3 SPAWNING
4 EGG MASSES
5 FRY TANKS
6 FINGERLING
7 R & R
MAIN LEVEL
SECTION

Figure 4. Concept of a polycultural wastewater reclamation aquaculture unit designed by architecture student Greg Gill. Simple concrete structure built into slope of rock outcrop at foot of reservoir. Basic design of hatchery implies water issuing forth from "womb of the earth" where embryonic fish are hatched, reared and eventually grow large enough to exit the hatchery into the external raceway culture units.

But times and attitudes have changed. Increases in population and per capita water consumption, coupled with an increased environmental consciousness, have led to increasing pressure for well-planned and controlled growth statewide. The Regional Water Quality Control Boards across the state have initiated a series of watershed basin studies which, when completed, will result in comprehensive water quality plans for each of 16 sub-basins. In the San Luis Obispo County coastal sub-basin, the interim plan of 1971 called simply for the discontinuance of effluent discharge into surface fresh waters before 1980[6]. The recommended plan now specifies land disposal with reclamation and reuse for all but two treatment plants[7]. In these two exceptions, better ocean outfalls are proposed but reuse and reclamation projects are still listed as potential alternatives.

Three years ago, substantial increases in overall agricultural irrigation development were not expected to occur within the county. Considerable increases in local citrus, avocado and wine grape acreages however have occurred. For example, along the coast an 8-fold increase, from 650 acres in 1970, to 5340 acres by the year 2000, is projected[8]. Similar increases are already occurring inland in The Upper Salinas Valley watershed with approximately 1600 new acres of vineyards in the past two years.

It is increasingly apparent, from the regional as well as local point of view, that as development of additional agricultural and urban land occurs, however well-controlled, some means of providing supplemental water, other than by importation, must be explored. Wastewater reclamation and reuse should have a high priority among them.

It has been said that wastes are resources out of place. In San Luis Obispo County, fully one-half of the 25,000 acre-feet of supplemental water needs projected for the year 2000[8] could potentially be met by reclaiming the relatively high quality wastewater being discharged from eleven existing secondary treatment plants (Table 1). The polycultural wastewater reclamation concept at Cal Poly evolved in part out of recognition of this economic and political reality.

But environmental forces were also at play. In 1971, one of the guidelines set by the Interim Water Quality Management Plan was that there shall be no effluent discharged into areas which possess unique or uncommon cultural, scenic, aesthetic, historical or scientific value[6]. Morro Bay, one of the least modified major estuaries on the

California Coast, is recognized as such a unique place. So it was that in early 1971, the Central Coastal Regional Water Quality Control Board gave notice to the California Department of Corrections, that the California Men's Colony treatment plant would eventually have to stop discharging into Chorro Creek, which flows into the Morro Bay estuary.

Fortuitously, at about the same time that the CMC received notice to consider land disposal of its effluent, officials of California Polytechnic State University, were becoming aware of production capacity problems in the two 200 + g.p.m. irrigation wells located on its Chorro Creek Ranch property. The university property consists of some 600 acres of valley floor adjacent to and downstream (west) from the prison's treatment plant (Fig.1). An interagency agreement was entered into on January 1, 1972 between both State agencies. Cal Poly was to take all it could use of the CMC wastewater effluent for land storage and agricultural irrigation. The remainder would still discharge into Chorro Creek.

Construction of a 50 acre-foot capacity reservoir was soon scheduled and the decision was made to build it the "Cal Poly way", i.e., with student class help under the direction of the instructional staff and faculty. The mechanics of building a new reservoir soon became intermingled with new ideas for aquaculture and catfish farming being investigated elsewhere on campus (see above). The process opened up the educational experiment described in the Introduction. It has already involved untold hours of labor from some 150 students and at least 25 faculty and staff members in 10 different instructional departments on campus. Four of the seven academic schools have been involved thus far.

SYSTEM DESIGN, FACILITIES AND PROPOSED STUDIES

Treatment Plant

The California Men's Colony wastewater facility is a secondary treatment plant consisting of barscreen, primary settling, biofilters, secondary settling, chlorination and sludge digestion apparatus. Design capacity is 1.5 m.g.d. Current average volume is 0.57 m.g.d. (Table 1). Average temperature of the effluent at discharge is 70°F. The CMC plant is located off State Highway 1, about halfway between the cities of San Luis Obispo and Morro Bay (Fig.1). It services the staff and prisoners of the Men's Colony, the staff and students of Cuesta College, and the staff of Camp San Luis Obispo (Army National Guard). There are no

Table 1. Current Disposition of Secondary Waste Water from San Luis Obispo County Treatment Plants[*]

PLANT LOCATION	DESIGN CAPACITY (m.g.d.)	AVERAGE[a] DISCHARGE (m.g.d.)	AVAILABLE RECLAMATION (a.f./yr.)	TYPE OF EXISTING DISPOSAL[b]
Coastal				
Cambria	0.25	0.06[c]	67	PP, SI
San Simeon	0.15	0.05[c]	56	OO
Cayucos/Morro Bay	1.70	1.10[c]	1232	OO
California Men's Colony	1.50	0.57[d]	638	FS
San Luis Obispo	5.00	3.99[e]	4368	EP,SI,FS
South County San.District	2.50	1.02[e]	1142	OO
Pismo Beach	1.00	0.30[f]	336	OO
Upper Salinas Valley				
San Miguel	0.30	0.30[c]	34	PP
Paso Robles	2.20	1.06[e]	1187	PP,FS
Atascadero	0.83	0.54[c]	605	PP,SI,FS
Atascadero State Hospital	0.50	0.14[c]	157	SI
TOTALS	15.93	8.86	9822	

a. Annual totals divided by 365
b. PP = Percolation Ponds; EP = Evaporation Ponds; SI = Spray Irrigation; FS = Freshwater Stream; OO = Ocean Outfall
c. Period January 1973 - December 1973
d. Period August 1972 - July 1973
e. Period July 1972 - June 1973
f. Period July 1971 - June 1972
* Basic data compiled from Central Coastal Regional Water Quality Control Board files, San Luis Obispo

industrial contaminants in the system, the prison laundry being the only source of sewerage in commercial proportions.

Chlorinated effluent of relatively good quality (Table 2) is discharged into Chorro Creek and it flows into Morro Bay estuary, about 4 miles to the west. Some percolation into the groundwater basin occurs. The City of Morro Bay obtains a portion of its domestic water supply from wells in the Chorro Creek basin downstream from the CMC discharge. These wells and others in the basin have been monitored over a period of years (Table 3)[9]. The maximum degredation of ground water shown in the tables has been the result of occasional sea water intrusion rather than from CMC effluent percolation.

Biological Treatment Lagoon (BTL)

Wastewater discharging from the CMC plant will be lifted by a 450 g.p.m., 15 h.p. pump some 60 feet in elevation and transported via a 6" P.V.C. pipe over a distance of 1500 feet to the newly-constructed 50 acre-foot capacity reservoir (Fig.1). There, primarily high nitrate uptake forage crops will be grown hydroponically in trains of floating racks. The young shoots of various grasses will be fed to campus livestock. A greenhouse will also be constructed for more controlled hydroponic experimentation.

Aquaculture Unit (AU)

Water from the BTL will flow by gravity directly to an existing 10 acre-foot capacity irrigation reservoir (Fig.1d) and/or through an experimental aquaculture unit of a design similar to those illustrated in Figures 3 and 4. The AU will consist of an experimental hatchery and dry labs, a parallel raceway system, and a series of small 0.1 acre ponds.

Experimental Hatchery - This will provide wet lab facilities for artificial spawning, hatching and rearing of warm or cold water fishes (Figs.3.4). Initial experimentation will utilize channel catfish, *Ictalurus punctatus*, with which we have had the most experience, and also the hitch, *Livinia exilicauda*, a native herbivorous fish from the Central Valley. We also hope to raise fresh water clams and local crayfish.

Dry Laboratory - This portion of the AU will feature a water and soil quality analysis lab (Figs.3.4). Computerized, remote multi-channel sensing equipment (Montedoro-Whitney Corp., San Luis Obispo) will allow continuous or

Table 2. Mineral Analysis and Effluent Characteristics, California Men's Colony Secondary Treatment Plant.

Characteristic	1970[a]	1971[b]	1972[c]	1973[d]	1973[e]
pH	7.6	7.7	7.8	7.6	8.2
ECx10^6@25°C (mmhos/cm)	-	-	1300	1500	-
Total Dissolved Solids [f]	672	955	-	1040	905
Total Hardness (as$CaCO_3$)	300	384	342	325	416
Total Alkalinity	255	458	-	370	377
Calcium (Ca)	28	22	29	18	21
Magnesium (Mg)	56	59	66	59	88
Sodium (Na)	108	160	190	205	197
Potassium (K)	8.0	9.2	5.4	9.0	10
Iron (Fe)	0.21	0.10	0.14	-	-
Carbonate (CO_3)	0	0	0	0	0
Bicarbonate (HCO_3)	255	458	318	370	-
Sulfate (SO_4)	32	53	66	58	44
Chloride (Cl)	158	233	291	258	243
Nitrate (NO_3)	17	50	11	18	21
Fluoride (F)	3.0	3.9	1.8	1.3	2.4
Orthophosphate (PO_4)	21	4.0	14.6	14	-
Boron (B)	0.19	0.4	1.36	0.25	-
B.O.D. Annual Mean (ppm)	-	-	6.0	9.8	-
B.O.D. Annual Range (ppm)	-	-	4-7	8-13	-
D.O. Annual Mean	-	-	7.1	7.2	-
Solids,Suspended (ppm)	12.4	5	40	9	-
Solids,Settleable (ml/l)	<0.1	<0.1	<0.1	<0.1	-
Grease	1.3	1.4	6.8	3.6	-
Turbidity (JTU)	-	-	9.5	25	-
Coliform (mpn)	≤45	≤45	≤45	≤45	-

a. CMC Ann. Report, Regional Water Quality Control Bd. Feb.16
b. " " " " " " " " June 11
c. " " " " " " " " July 18
d. " " " " " " " " July 11
e. Regional Water Quality Control Bd.sample obtained June 27
f. Constituents listed in mg/l unless otherwise specified.

Table 3. Summary of Selected Mineral Constituents in Component Portions of Chorro Creek Basin Water[a].

		SURFACE WATER	GROUND WATER	CMC EFFLUENT
No. Analyses		12	90	16
No. Wells Tested		-	23	-
Mg	Avg.	68	114	-
	(Range)	(27-103)	(9-436)	-
SO_4	Avg.	31	104	91
	(Range)	(0-59)	(14-788)	(29-589)
Cl	Avg.	43	254	272
	(Range)	(19-77)	(45-2559)	(114-865)
NO_3	Avg.	4.1	14.5	23
	(Range)	(0-9.9)	(0-105.0)	(0-98.0)
B	Avg.	0.09	0.13	-
	(Range)	(0-0.20)	(0-0.71)	-
TDS	Avg.	433	1022	1045
	(Range)	(234-704)	(547-5402)	(766-1997)
EC x 10^6 @ 25°C	Avg.	685	1643	-
	(Range)	(347-1080)	(990-8264)	-

a. Abstracted from Tables 9, 10, 14 - Reference No. 9

periodic monitoring of several water parameters at critical locations in the circulation pathway, including monitor wells around the crop irrigation area. Additional facilities will include large capacity aquarium units with room for observation of the natural reproductive behavior of catfish and other warm-or coldwater species. A pathology lab will be set up for studying and monitoring fish diseases and to investigate the transmission of microbial pathogens from the effluent water.

Since one of the functions of our project is to educate the public, the architectural design takes into consideration not only the needs of the students and faculty, but also the visitor. Guided or self-guided tours of the facilities will be designed to educate without disrupting routine operations.

Irrigation Reservoir

Wastewater from the BTL and the AU will exit to the 10 acre-foot capacity irrigation reservoir (Fig.1d). Outflow water from this storage site will be used to irrigate field crops in the crops area west of the reservoir site (Fig.1e).

Crops Area

The areal geology of the location indicates an upper Pleistocene alluvium of sand, gravel and clay. The slopes and mounds are of Jurassic Franciscan formation consisting of sedimentary and igneous rocks. The creek terrace soils are high in clay content with low permeability and slow rate of infiltration.

Studies similar to those done at the Penn State University Waste Water Renovation Project[10] will be undertaken. Faculty and students from the Soils Science Department will analyze soils and monitor the crops area before, during and after effluent is utilized for irrigation purposes. Deep cores will be taken and test plots utilizing wastewater will be compared with those using well water.

Faculty and students from the Crops Science Department will study yields and quality of field crops under irrigation with wastewater and well water.

Sludge collected from the fish raceway system will be analyzed and utilized as fertilizer or for methane gas generation. Sludge from the CMC treatment plant will also be evaluated for its potential fertilizer value. Terrestial and hydroponic crop wastes not otherwise utilizable will be composted along with sludge.

Additional Studies

Utilization of Other Wastes - High protein content fish carcasses, from the Morro Bay commercial fisheries, are currently being disposed of by land burial. We propose to convert this valuable waste into fish meal. We will combine this with high protein material gathered from terrestial or hydroponic crop wastes. Aquatic crops of Wolffia or Lemna spp., which are indigenous to our county, will also be propagated in our ponds. With an appropriate vitamin mix added, we intend to produce a viable pelletized fish food at our campus feed mill. With formula variations, we hope to use it for other campus livestock.

Irrigation and Erosion Controls - Faculty and students from the Agricultural Engineering Department will design the irrigation systems, erosion control systems and the overall hydraulics of the system.

Solar Heating - Faculty and students from Architecture and Environmental Engineering Departments will design a roof or wall panels for the aquaculture unit which will feature solar powered heating of either well water or wastewater for experimental use in the hatchery.

Food Processing - Faculty and students in the Food Industries Department will study the technology of processing, packaging and freezing fish products.

We hope to make our polyculture farm a truly multidisciplinary educational unit where faculty can conduct research, where students can learn by doing, and where the general public can be exposed to graphic examples of necessary technologies.

CONCLUSIONS

We believe it is possible to make a resource out of wastewater by routing it through a series of aquatic and terrestial biotic filters. We believe that known methods of hydroponic culture, aquaculture and agriculture can be combined into a polycultural process which is not only economically feasible but also ecologically desireable. Furthermore, it is our responsibility as educators not only to teach our students but to educate the public at large.

SUMMARY

Only a small percentage of secondary wastewater is being reclaimed or reused in Southern California.

Regional Water Quality Control Boards have adopted a policy of disposal on land for reclamation or reuse with a goal of substantially reduced ocean outfalls.

It is suggested that wastewater reclamation and reuse should have high priority among alternate means of supplying supplemental water in San Luis Obispo County, especially in areas where overdrafting and mining of ground water has occurred. There is a need for considerable consumer education.

The Cal Poly project will utilize secondary effluent from the California Men's Colony wastewater treatment plant to grow harvestable crops hydroponically, aquaculturally and agriculturally. It is designed for applied research, student training and public education.

The aquaculture unit wet labs will facilitate the study of reproductive behavior, hatching and rearing of warm or cold water fishes. The dry lab portions will be equipped to study pathology of fishes and other organisms growing in the secondary effluent. Comparative analysis of soil and water will occur and remote monitoring of both will be possible at various critical points in the circulation pathway.

The student-teacher interaction is a vital component of this interdisciplinary academic instructional system at Cal Poly.

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PART 7 ADDITIONAL PAPERS CONTRIBUTED FOR PUBLICATION

COLIFORM AND PHYTOPLANKTON STUDIES IN A BRACKISH WATER AQUACULTURE POND FERTILIZED WITH DOMESTIC WASTEWATER

by

Robert F. Donnelly and Tommy T. Inouye*

INTRODUCTION

Two aquaculture ponds, of about 0.15 hectares each, were constructed within the confines of the City of Arcata sewage lagoon, located at the north end of Humboldt Bay, California. These two ponds were placed in operation June 1971. One pond (designated North Pond) was the experimental site and was filled with a 50-50 mixture of seawater and sewage effluent from the sewage lagoon. The other pond (designated South Pond) was filled with seawater only and used as the control in some experiments. Funding for construction of the ponds came from the Wildlife Conservation Board of California and operating monies were provided by the California State University, Humboldt, Coherent Area Sea Grant Program of the National Oceanographic and Atmospheric Administration, U.S. Department of Commerce.[1]

No standards were available for sewage-seawater pond discharge; therefore, the Public Health Service indicated that a minimum of 2 weeks retention was needed in the North Pond before the water could be discharged into Humboldt Bay without chlorination. As a result of this and other considerations, it was decided that one of the authors (Robert Donnelly) would conduct a study of the coliform levels of the two ponds, the sewage lagoon itself, and Humboldt Bay, adjacent to the aquaculture ponds. Concurrently, Tommy Inouye undertook a study of phytoplankton identification and enumeration of the same water samples used for coliform determinations. At the same time, six other variables, (pH, DO, salinity, temperature, Secchi disc readings, and depth), were collected. Due to the large number of samples collected (192), only the 48 samples from the North Pond were analyzed for their phytoplankton content.

This paper discusses the possible interrelations among these variables. Several authors,[2, 3, 4, and 5] have demonstrated relationships between phytoplankton and bacteria. The bacteria break down organic material and make it available for phytoplankton utilization and, in turn, the

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phytoplankton give off organic materials and contribute dead cells to the bacterial substrate. Some of our data appear to support this cycle.

MATERIALS AND METHODS

LOCATION AND TIME OF SAMPLING

Concurrently, samples of coliforms and phytoplankton were taken from the North Pond during two different seasons of 1972 (April 15 through May 27 and September 27 through October 31).[6] Collections were made adjacent to the pond outlet (headgate) six times during each season at approximately 1-week intervals. Four depths were sampled each time. The surface sample was actually 20 cm below the surface, while the bottom sample was taken 10 cm above the bottom. The two intermediate sample depths were equally spaced between these surface and bottom depths. The water level fluctuated due to rain and evaporation, resulting in different absolute sampling depths each week.

SAMPLING DEVICE

The sampling device consisted of a 500-ml aspirator bottle, a sterilized and partially evacuated 125-ml bottle, each attached to a long handle, (Figs. 1, 2, and 3).

Five ml of water and a few boiling chips were placed in each 125-ml sample bottle. The bottles were stoppered with foam stoppers. Rubber stoppers of appropriate size were drilled to accept a Pasteur pipette. The Pasteur pipettes were bent and flame closed, and then inserted into the rubber stoppers (Fig. 1).

The rubber stoppers with the bent Pasteur pipettes and foam-stoppered bottles were autoclaved. After sterilization, the bottles were placed over a flame and the water within was allowed to boil for a short period of time. The bottles were removed from the flame and, using aseptic technique, the rubber stoppers with bent Pasteur pipettes were inserted into the mouth of each bottle. These were allowed to cool, creating a partial vacuum. Experimentation showed that these partially evacuated 125-ml bottles would obtain a sample of about 90 ml when the bent Pasteur pipette was broken beneath the surface of the water. The inside diameter of the Pasteur pipette (about 5 mm) was small enough so that the sample water was held within by surface tension.

The aspirator bottle was controlled through a small plastic tube running from the top of the bottle to the operator. The sterilized 125-ml bottle was activated by an attached pole struck by a hammer (Fig. 3).

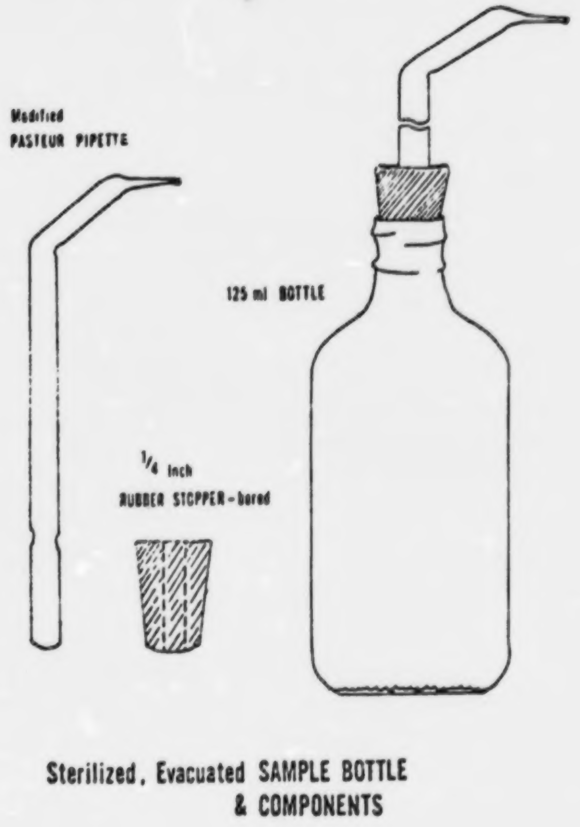
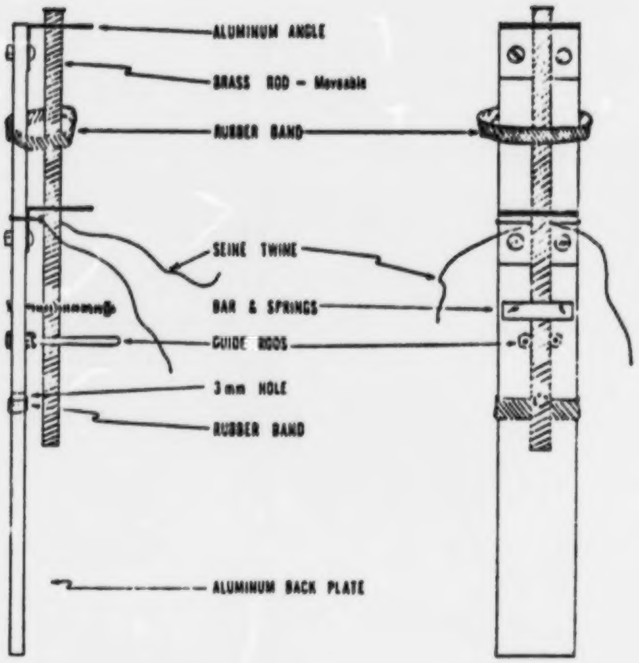

Modified
PASTEUR PIPETTE
125 ml BOTTLE
1/4 inch
RUBBER STOPPER-bored
Sterilized, Evacuated SAMPLE BOTTLE
& COMPONENTS

Figure 1. 125-ml sterilized water sample bottle (complete) bent Pasteur pipette and bored rubber stopper.

TREATMENT OF THE SAMPLES

Water collected with the aspirator bottle was used to determine DO, pH, salinity, and temperature at the sample site. The 125-ml samples were processed at the laboratory where approximately 35 ml of each sample was inoculated into the nine-tube MPN test for coliforms. The coliform determinations were made according to the procedure outlined in Standard Methods.[7] One Secchi disc measurement was taken on each day sampled.

The remainder of the 125-ml sample was inoculated with iodine (1 drop


ALUMINUM ANGLE
BRASS ROD - Moveable
RUBBER BAND
SEINE TWINE
BAR & SPRINGS
GUIDE RODS
3 mm HOLE
RUBBER BAND
ALUMINUM BACK PLATE

SAMPLING DEVICE for ACTIVATING
Sterilized, Evacuated Sample Bottle

Figure 2. The device used for activating the sterilized and partially evacuated 125-ml bottles.

per 10 ml of sample) for phytoplankton identification and counting. This was accomplished with an inverted microscope and hemocytometer counting chamber.

RESULTS AND DISCUSSION

The data were analyzed by BMDO2R computer program in two ways: 1) by 6-week time periods, and 2) some of the 12-weeks of data were pooled and analyzed. The first 6-week sampling period included a period after the sewage-seawater mixture had been in the North Pond for approximately 2.5 months, while the second 6-week period started 2 days after a sewage-seawater mixture was introduced into the pond. The coliform levels

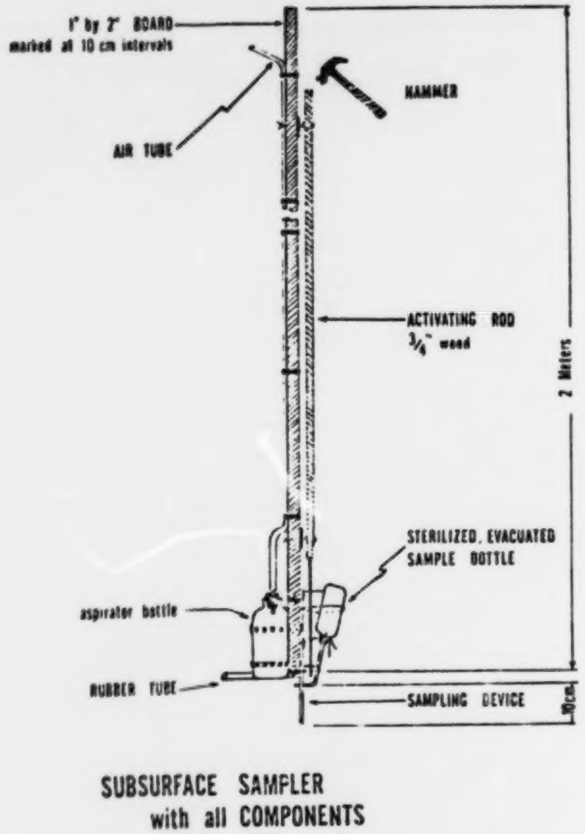

1" by 2" BOARD
marked at 10 cm intervals
HAMMER
AIR TUBE
ACTIVATING ROD
3/4" wood
2 Meters
STERILIZED, EVACUATED
SAMPLE BOTTLE
aspirator bottle
RUBBER TUBE
SAMPLING DEVICE
10cm
SUBSURFACE SAMPLER
with all COMPONENTS

Figure 3. Assembled apparatus for obtaining subsurface water samples.

remained fairly low during the first period, while the levels started high and decreased until more water was added from Humboldt Bay during the second sampling period (Fig. 4).[6] Humboldt Bay generally had higher coliform levels than the North Pond.

This second period is of interest because we wanted to know what was occurring in the water column during the time the pond was undergoing its greatest developmental changes. After the pond had stabilized, large fluctuations in organism populations no longer occurred.

Three genera of phytoplankton were found: Anacystis, Ankistrodesmus,

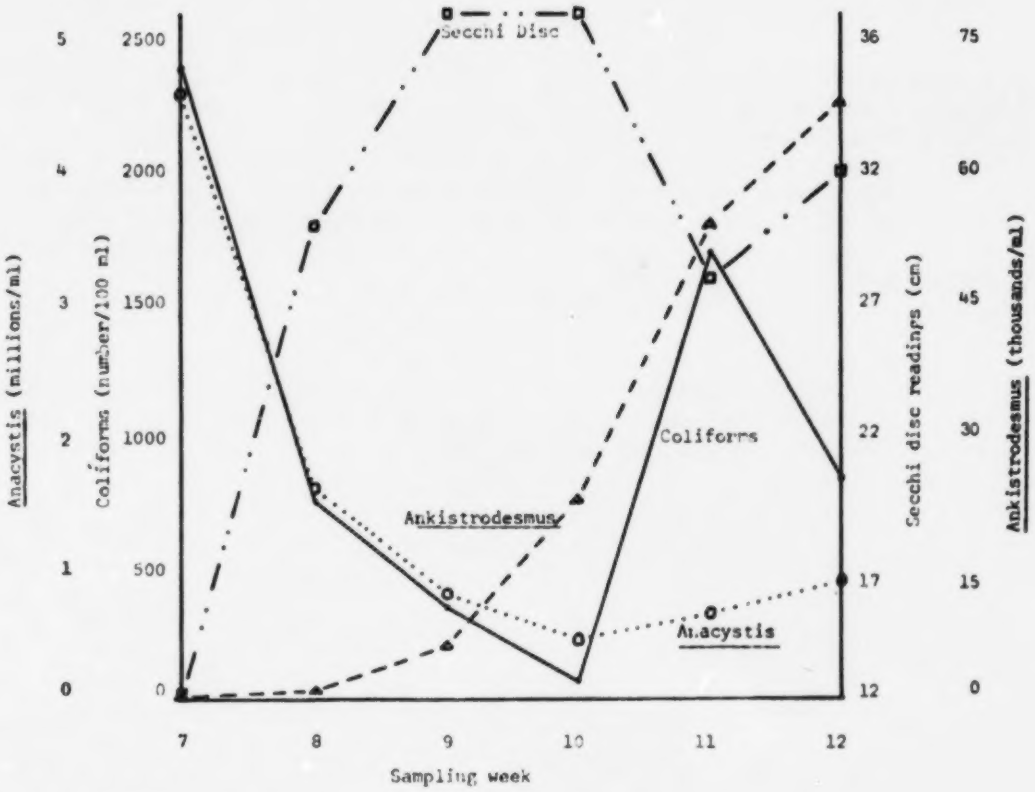


Figure 4. Anacystis numbers, coliforms levels, Secchi disc readings and Ankistrodesmus numbers (averages of the four depths) during the second sampling period from September 27 to October 31, 1972. Note the sharp rise in coliform levels from week 10 to 11 is apparently due to an injection of Humboldt Bay water on October 23.

and Gymnodinium, with Anacystis being most abundant and Gymnodinium least numerous. A negative correlation existed between coliform levels and Secchi disc measurements and between coliform levels and Ankistrodesmus numbers (Fig. 4). A positive correlation existed between coliform levels and Anacystis numbers (Fig. 4). All other variables showed no significant correlation.

CONCLUSIONS

The data appears to support the hypothesis that there is a direct relationship between Anacystis and coliforms. However, a negative correlation existed for Ankistrodesmus and coliforms. One study demonstrated that bacteria utilize the organics secreted by phytoplankton.[4] In another study, phytoplankton was shown to utilize inorganic nutrients generated from bacterial breakdown of organic materials in lakes.[3] This leads us to believe that there may be a nutrient interrelationship between Anacystis and coliforms.

ACKNOWLEDGMENTS

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MINERAL QUALITY OF FISH POND EFFLUENT RELATED TO SOIL PROPERTIES AND CROP PRODUCTION

by

L. H. Hileman*

INTRODUCTION

Commercial fish production in Arkansas is a $21,000,000 industry and a steadly growing enterprise. Catfish are efficient converters of feed into edible meat high in protein, therefore, a valuable source of human food. Farm production is carried out in earth ponds varying in size from 10 to 20 or more acres usually filled with water pumped from underground aquifers. Where adequate water is available the fish are grown in a system of flumes referred to as raceways which allows for continous water movement. Water from these catfish production areas, whether ponds or raceways, is considered as waste water. A large amount of this waste water is discharged into streams, and rivers. Enforcement of regulations pertaining to the discharge of water into streams and rivers caused fish farmers to consider other methods of disposal. A logical consideration would be the use of this waste water for crop irrigation. A large number of catfish farmers also grow other food or fiber crops such as soybeans, rice or cotton. Previous water analysis parameters were concerned with quality factors affecting municipal or industrial uses. The use of waste water for irrigation may have little direct effect on the growing crop but can drastically effect soil chemistry thus, indirectly affecting crop production over a long period of time. Certain irrigation water quality parameters will be discussed, and also data obtained from the chemical analysis of catfish waste water related to potential effects on the soil and plant growth.

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RESULTS AND DISCUSSION

Irrigation water quality is determined by the kind and amount of mineral elements present in the water, and the relationship of these elements to the soil's chemical and physical properties as they affect both immediate and future crop production. Frequently, in practice the major concern is with the immediate effect on the growing crop while the most damaging to the farmer are the long term effects on the soil. Emphasis in this paper will be placed on the long term effects on the soil and their relationship to plant growth.

Data in Table 1 shows some of the important parameters for determining the quality of water for irrigation. Water containing relatively high amount of calcium and magnesium when applied to soils will, in time, cause a condition known as a saline soil. A saline soil is one that is high in soluble calcium and magnesium. Such soils are "salty" due to the accumulation of these salts near the surface. The principal effect of high soluble salts is to reduce the availability of water to the plant. A high soluble salt level will also interfer with the plant uptake of other needed ions such as potassium, nitrogen, and iron. The calcium and magnesium levels found in catfish waste water were quite low, (Table 1). It is interesting to note that the water was initially rather high in calcium and magnesium (5.1 meq/l) but this level declined rapidly and reached an equilibrium of near 0.9 meq/l during the growth period. Average calcium plus magnesium data for seven locations shows low values indicating that no serious soil salinity problems would be expected when any of these waters were used for irrigation.

Irrigation water containing high amounts of sodium creates a very serious soil problem known as sodic or alkali soils. These soils are usually strongly alkaline in reaction, (pH 8.5 to 10.0) dispersed, almost impermeable to water, and have poor tilth. This results in reduced plant growth because of inadequate water penetration, poor root zone aeration and soil crusting. The large amount of sodium in these soils also interfers with the plants nutrition by preventing the uptake of potassium, calcium, and magnesium as well as many of the micro-nutrients. It is almost impossible to reclaim sodic soils that have restricted internal drainage. Data in Table 1, shows that this water was very low in sodium through the first six sampling periods but then showed a decided increase. Sodium values as shown in Table 1 are not considered high, however, the more important factor is the proportion of sodium to calcium and magnesium. This relationship is expressed by the S.A.R. and the S.S.P. values which are shown in Table 1. The S.A.R. or sodium adsorption ratio is high when it exceeds 10.0 for soils with good internal drainage and above 5.0 for soils with poor internal drainage. The average S.A.R. for location 1 is 3.4 (Table 2), which is less than 5.0. The S.A.R. is based on the soil exchange system which may respond differently under adverse conditions. The soluble sodium percentage, S.S.P. is the soluble sodium as a percent of the total soluble cations (Ca, Mg, Na, K) in the irrigation water. S.S.P. values exceeding 60 are usually considered high for clay soils with impeded internal drainage. The water from location 1 would be considered useable based on the S.A.R. while it would be considered unsatisfactory based on the S.S.P. The author's experience in Arkansas indicates that the S.S.P. is the more valid for Arkansas soil conditions. Locations 1, 3, 4, and 6 have high S.S.P. values while locations 8, 9, and 10 are much lower, Table 2.

The data in Table 2 shows the location variation that can occur with respect to the irrigation quality of catfish production waste water. The results of indescriminate use of these waters can result in the alteration of plant nutrient uptake, therefore, altered plant quality, reduce crop yields, and soil physical and chemical damage. Farmers planning to use catfish waste water for irrigation should obtain a chemical analysis and irrigation quality evaluation before applying it to their soils.

All of us should keep in mind the thoughtful statement of Franklin D. Roosevelt; "The history of every nation is eventually written in the way in which it cares for its soil".

Table 1, Calcium, Magnesium, Sodium, Bicarbonates, Total Dissolved Salts, S. A. R. and S. S. P. Values for Catfish Production Water at Location 1, 1971 for the Two Week Sampling Periods.

Sampling*	Ca +Mg	Na meq/l	HCO_3	T. D. S. ppm	S. A. R. ratio	S. S. P. %
1- fill	5.1	0.5	3.8	269	>1.0	8.9
2	1.9	0.5	4.2	307	>1.0	20.8
3	0.7	0.5	4.2	294	1.0	41.6
4	0.8	0.5	4.3	320	1.1	39.2
5	1.4	0.1	7.0	64	>1.0	6.6
6	0.7	0.05	4.1	282	1.0	6.6
7	0.6	3.5	3.8	283	6.8	85.4
8	0.9	4.1	3.8	269	6.0	82.0
9	0.7	3.9	3.9	269	6.1	84.8
10	0.6	3.6	3.6	256	7.1	85.7
11	0.7	3.7	3.7	269	6.0	84.0
12	1.1	3.6	3.9	269	>1.0	76.0
13	0.8	4.0	4.0	307	6.0	83.3
14	0.8	3.5	4.2	294	5.6	81.4
15	0.7	0.05	4.0	307	1.0	6.6
16	0.6	0.5	4.3	320	>1.0	45.4
17	0.8	4.1	4.5	320	5.8	83.6
18	0.9	0.4	3.8	269	1.3	30.7
19	0.8	3.9	3.8	269	6.0	82.9
20	0.8	3.8	3.6	269	6.1	82.6
21	0.8	3.7	3.0	243	5.6	82.2
22	0.8	3.5	3.2	243	5.3	81.4
23	0.8	2.2	2.8	192	3.8	73.3
24	0.8	1.4	3.1	256	2.4	63.6
25	0.9	3.2	3.5	269	4.8	78.0
26	0.8	2.3	3.6	269	3.8	74.2
27	0.8	2.6	3.6	243	4.8	76.5
28	0.9	2.7	3.1	230	4.0	75.0
29	0.9	2.9	3.0	243	4.6	76.3

* Samples taken at two week intervals.

Table 2. Average Calcium, Magnesium, Sodium, Bicarbonate, Total Dissolved Salts, S.A.R. and S.S.P. Values for Catfish Production Waters at Several Locations.

Location*	Ca+Mg	Na meq/l	HCO_3	T.D.S ppm	S.A.R. ratio	S.S.P. %
1	0.99	2.4	3.8	265	3.4	70.6
3	0.58	4.1	3.0	405	7.6	87.2
4	0.86	2.7	3.3	269	3.8	75.0
6	0.53	4.3	2.8	383	9.0	89.6
8	1.60	0.8	1.9	221	1.0	33.0
9	1.13	0.5	1.6	145	1.0	31.2
10	1.60	0.9	2.1	226	1.0	36.0

* 1, 3, 4, and 6 were in the Coastal Plain of Mississippi. Locations 8, 9, and 10 were in the Mississippi Delta of Arkansas.

THE DIALECTICS OF A PROPOSAL
ON
BIOLOGICAL CONTROL OF EUTROPHICATION IN
SEWAGE LAGOONS

by

S. Y. Lin[+]

ABSTRACT

The existing lagoons for the deposition of municipal sewage face the problems of extreme eutrophication and of the effluents being highly polluted for discharge into public waters. An elaborate mechanical engineering plant if established to purify the sewage water would be very expensive in both initial cost and maintenance. The present proposal is to take the advantages of biological control by utilization of natural performance of pure plankton feeding fishes, filamentous algae eaters, bottom feeders and scavengers to purify the sewage water. It is more economical this way than any mechanical engineering design. In addition biological control has many advantages in fitting in the principles of environment improvement and protection and in production of animal proteins.

INTRODUCTION

BACKGROUND INFORMATION AND GENERAL CONSIDERATIONS

The formulation of this dialectics is the result of many long discussions with J. M. Malone Jr., Manager of J. M. Malone and Son Enterprises of Lonoke, Arkansas, and after some observations made on the sewage lagoons in Lonoke and England, Arkansas.

The most common phenomenon in these sewage holding lagoons is the process of eutrophication as a result of the growth of phytoplankton which will reach in summer warm days to the highest level through the support of the organic and mineral nutrients coming in with the domestic sewage. However eutrophication will not

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occur if the domestic sewage is too thick with sludge or mixed with industrial waste, some substances from which will kill the algae. Otherwise at the highest level of eutrophication no more space and dissolved oxygen are available for further expansion; meanwhile toxic substances secreted by the animals and plants are accumulated to so high a concentration that the algae and zooplankton can no longer survive resulting in total death of most of the biota in the lagoon followed by decomposition to produce the worst situation of pollution which may last for a considerable length of time before the lagoon can return to suitable conditions for the growth of phytoplankton again. This is locally known as 'a turnover. It is a biological turnover, to be sure, but it must be distinguished from the limnological turnovers during the spring and fall due to thermal difference and wind action. Under the present circumstances the effluents from many such highly polluted lagoons are being directly discharged into public waters.

For this reason alone it is highly desirable if such excessive eutrophication can be prevented by introduction of certain plankton feeders to graze down the exuberant algae so as to avoid a turnover and moreover a system of new ponds be built to purify the effluents through similar biological process. This so far as dialectics go can be satisfactorily achieved.

While eutrophication is advancing, not only the planktonic algae are in exuberant bloom but also zooplankton, bottom worms and algae, snails and insects are thriving to the highest degree of abundance. So in addition to the introduction of phytoplankton and zooplankton feeders, omnivores of bottom feeding habit and even scavengers like common carp and carpsuckers which consume waste organic matter in suspension and on the bottom are necessary to be added to the sewage ponds so that a full balanced extent of biological control can be accomplished. Naturally the fishes so introduced will produce reasonable amount of organic waste in the form of faeces, but such excreta which have been partly digested and fermented in the digestive tract of the fish are easily and rapidly decomposed by bacteria into simple gases, water, minerals and soluble organic substances.

Actually what happen in the sewage lagoon and the ponds is a series of transformations of inorganic components activated by the energy of the sun in the phytoplankton into organic matter and then back to inorganic components by animals and bacteria. The first transformation, of course, involves the process of photosynthesis in which the mineral nutrients are taken up by the phytoplankton to form organic substances with CO_2 and H_2O as basic materials of life. Next come the zooplankton, worms, molluscs insects and fish to feed on the phytoplankton, but as the population of the phytoplankton is so large, these animals can only consume a small portion of it. In the third phase the large animals will prey on the small ones. At last when the plants and animals die their bodies will be decomposed by bacteria to simple organic and mineral components, H_2O and CO_2 which the algae will be able to utilize again.

In the instance when no efficient plankton feeding fishes are present, the phytoplankton and zooplankton will increase rapidly to the highest degree with a consequence of total death of the planktons caused by population explosion shock. But with the introduction of the phytoplankton feeding fishes to graze down a substantial part of the phytoplankton and to keep the biological community in balance, a turnover would be prevented as illustrated in figure 1.

Now let us review some of the information in literature as how far this principle of biological control has been applied in the field of fish culture with the purpose to improve wildlife environment in rivers and lakes and to increase fish production in ponds.

REVIEW OF LITERATURE

There are many kinds of aquatic animals which feed on planktonic algae. Oysters, clams and mussels, for example, are effieient algae consumers. Based on this feeding habit Ryther _et al_ (1972) envisaged the possibility of using tertiary sewage for oyster culture under cotrolled eutrophication: He offered a very interesting dialectics in which he suggested that a town of 50,000 people could, with its 51 ha aquaculture-sewage treatment system, raise an annual crop of over 900 tons of oyster meat or nearly 90,000 hectoliters (250,000 bushels) of whole oysters worth, in today's market as a luxury table oyster, upwards to $5 million. But upon further search of information it is revealed that a certain fishes because of their large size and free swimming habit may be even more adequate for the control of phytoplankton. As the studies of Lin(1969) indicated, the silver amur or silver carp (_Hypophthalmichthys molitrix_) and the bighead (_Aristichthys nobilis_) which grow to 10 or 15 kgs in a few years under favorable conditions are pure and efficient plankton feeders. The silver amur because of its spongy-plate-like gill-rakers that can filter minute particulates in water feeds principally on macrophytoplankton as well as microphytoplankton, while the bighead with closely set but separate gill-rakers filters out only the macrophytoplankton and zooplankton for food.

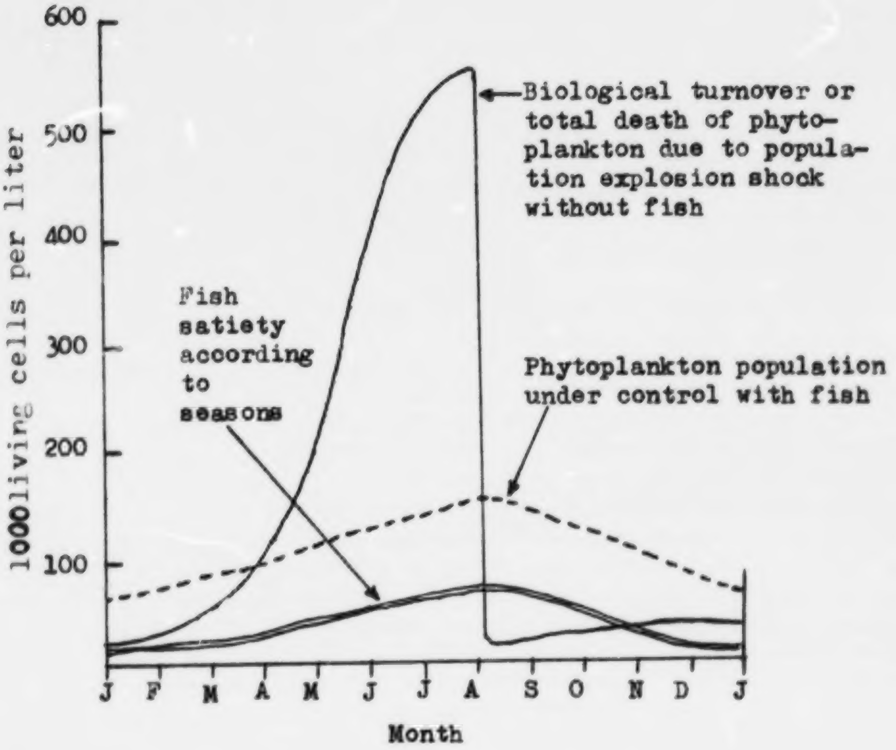

Figure 1 - Hypothetical curves of phytoplankton population trends in sewage lagoon before (solid line) and after (broken line) the stocking of fishes (double lines).

Mukhamedova and Sarsembayev (1967) found by experiments that silver carp fry of 1.5 g consumed food equal to 17% of its body weight and the young of 5.8 g 12% and Omarov (1970) carried out experiments further on the feeding patterns of silver carp weighing 328 to 380 g and revealed that the fish ingested food in 24 hours at 23°C with a DO of 4.23 ml/l of 19% of its body weight on the average. At higher temperature (e.g. 26°-30°C) the consumption rate will certainly be increased. Apart from this information we are not aware so far that there have been any comprehensive studies on the quantity of plankton being consumed by the silver carp per unit body weight per day under conditions of different seasons. Information on the quantitative feeding of the bighead is entirely lacking.

It has been observed, however, that the grass carp (Ctenopharyngodon idella) known as pure herbivore feeding on macrophytes can consume aquatic weeds 50 to 100 or even 200 (as claimed by some fish farmers) per cent of its body weight a day dependent on the size of the fish and water temperature. This means that a large fish would eat comparatively more weeds than a smaller one. Here we may assume that both the silver carp and bighead would consume phytoplankton 20 to 50 per cent of their weight per day in water temperature 26° to 30°C. It follows that if a lagoon is stocked with 3000 kg/ha of the two fishes each more than 500g, then 600 to 1500 kg/ha per day or 120 to 300 tons in 200 days chiefly of phytoplankton could be consumed by the fishes a year. It is estimated that under optimal conditions a freshwater pond or a sea water lagoon has the potentiality of producing 160 or 200 tons respectively of phytoplankton per ha/year (Tamiya, 1957 and Ryther et al, 1972). In the case of sewage lagoon if a quarter to half of this production could be removed, probably a biological turnover would be avoided and this may be effectively accomplished by rational stocking with the above mentioned fishes together with black carp (Mylopharyngodon piceus) and some other bottom algae and worm feeders such as grey mullet (Mugil cephalus) and mud carp (Cirrhinus molitorella) also.

It is significant that Cyanophyta constitute the major part of food bolus (over 60%) in the digestive tract of silver carp (Lin, 1969 and Chiang, 1971) and for this reason the fish will be an effective agent for controlling the blue green algae which are always abundant in the sewage lagoons. In China over two million ha of freshwater ponds are in existence; many of them were built centuries ago. Most of them especially those lying in the Pearl River and Yangtze River deltas are heavily fertilized with domestic sewage, animal manure plus supplementary feeding and are stocked with silver carp, bighead, grass carp, black carp, mud carp and common carp (Lin, 1954; Hickling, 1971; and Chinese

Fish Culture Experience Committee, 1961). As a result of such polyculture excessive eutrophication is always placed under control and consequently the so-called biological turnover very seldom occurs.

The carppond complex at Munich and Berlin serving as sewage purification fields is well known (Schaeperclaus, 1961 and Hickling, 1971). After the sewage effluent is diluted by 3 or 4 to 1 with freshwater it is sprayed into ponds where the mixed effluent is purified through natural process and can be safely discharged into rivers.

The use of undiluted domestic sewage waste water from a sewage treatment plant at Kielce, Poland, for the culture of Crucian carp (Carassius carassius) with satisfactory results is even more impressive (Thorsland, 1971). The effluent from the treatment plant (by activated sludge method but without clorination) was led into five experimental ponds, three of which were stocked with carp and two as control. Fish yield without supplementary feeding was up to 1,317 kg/ha, the highest yield ever obtained in Poland. Allen (1970) advocated the constructive use of sewage water for fish culture; Huggins (1970) and Konefes (1970) experimented on the use of tertiary treatment ponds to raise channel catfish and fathead minnow with encouraging results. Tsai (1973) studied fish sensitivity in sewage polluted streams of Maryland with results hinting strongly on the potentiality of using sewage waste water for fish culture.

OBJECTIVES OF THE PROPOSAL

In trying to solve the problems of eutrophication and that of the purification of the effluents coming out of the sewage lagoons it is considered necessary to test the hypothesis or dialectics in the best practical manner possible and thereupon it is proposed to initiate a pilot project with the following objectives in view:

1. The project will be designed to develop different patterns of fish communities each species in which will perform a distinct function in controlling a certain element or elements in the sewage waste water. The combined functions of different species should eventually result in purification of the sewage water.

2. It is further designed to test whether aquatic plants in combination with fishes would enhance the purification process.

3. Meanwhile the feeding habits of the silver amur and bighead will be studied and determined quantitatively. This knowledge is particularly important in forming a basis for stocking rate design.

4. The project would provide sufficient data to show ever more strongly that sewage waste water constitutes a great potential source for fish production which could be utilized for both human and animal food.

A PROPOSED PILOT PROJECT

With the above objectives in view and on the basis of the information discussed before it appears very feasible that the experiences and results obtained in the past can be applied to the management of the sewage lagoons in the United States with a good chance of success. After a preliminary survey of the sewage lagoon system in Lonoke and England, Arkansas, we are convinced that a great deal can be learned from a pilot project and therefore we strongly feel that a project of this nature should be started as soon as possible.

SITE FOR THE PILOT PROJECT

It is proposed to use the sewage lagoon of Lonoke or any other town or city for the establishment of the pilot project in the first year. When it proves a success after a period of test, one or two or even three other lagoons may be selected for next period's test. In the third period, some three or four years later, if the results of the first two period's experiments are satisfactory, work will be extended to as many lagoons as practical.

The sewage lagoon of Lonoke has a superficie of eight hectares (20 acres) and a depth of 1.5 meters. It receives an inflow of domestic sewage at the rate of some 500,000 gallons a day from a population of 4,000 people. On June 3, 1973 the lagoon was observed to be at a high level of eutrophication but not up to the stage of turnover. Anacystis and other blue green algae were abundant; some small fish (probably Gambusia) were seen along the margins of the lagoon and common carp were reported also to be present.

SIGNIFICANT ADVANTAGES OF THE PROJECT

Although it would be difficult to make any precise evaluation of the project at this moment its implementation would obviously have many advantages: (1) It is an economical project. The cost of construction and maintenance of any mechanical engineering sewage treatment plant will be always higher than this proposed biological control plant. Roughly speaking the establishment of amechanical engineering plant may cost over a million dollars while that of a biological control plant will cost only half or even less as much. The maintenance cost will be also much more in the engineering plant than in a biological control plant; (2) if the purification ponds are to be well arranged and constructed with roads and trees are planted around, no smell will be emitted from the lagoon and ponds and such area may well become a suitable spot for recreation purpose; (3) the sewage purification pond system when well stocked with fish can be open to public for sport fishing; (4) fish is one of the principal sources of animal protein for human consumption. If all the sewage lagoons in the United States are to be placed under similar management fish crop would be considerable, which can be rationally harvested and processed to serve as animal and human food; (5) the project would turn all the hazards of the domestic sewage ponds into a natural purification system that would be in perfect harmony with any balanced environment.

ORGANIZATION AND MANAGEMENT

Any private fish farm in the United States which has the facilities and expertise to propagate and cultivate the silver amur, the bighead, the black, the white amur and the common carp can be trusted by contract by a city or town government to manage the project. Such private fish farm under contract will take the responsibility to design, develop and maintain the pilot project. At the request of the city government the Environment Protection Agency of Federal Government should subsidize the Project by grant funds.

An essential task of the private farm as a managing agent is to propagate all the fishes required to stock the biological control sewage system and to replace the fishes after their removal at harvestable size.

When this pilot project proves a success at the end of a year or so, it is envisaged that work would be continued and extended to other city sewage systems under similar working and organization relationship and financial arrangement between EPA, city government and the private fish farm as managing agent.

METHODS AND PROCEDURE

CONSTRUCTION OF PURIFICATION PONDS

The first step to be taken for the construction of the purification ponds is to make a topographic survey of the area concerned and then basing on the topographic map a pond system will be designed and constructed.

As a usual practice one hectare of sewage lagoon is provided for a population of 500 people. For example, for a town of 4,000 people like Lonoke of Arkansas, a lagoon of 8 ha or 20 acres at 200 persons per acre with about 1.5 meter in depth has been built. Despite the lack of data it is assumed that the concentration of the nutrients in the secondary effluents from the sewage lagoon ranges quite close to the national average which shows in micron moles per liter: 242 NO_3, 1180 NH_4, 264 PO_4, and 656 SiO_3 (Ryther _et al_, 1972). Concentration of such level is, of course, still too high for discharge into public waters. In order to reduce the concentration it is propos ed to construct six ponds each one ha in superficie and two meters in depth, below the existing sewage lagoon of 8 ha to receive the effluent for further purification, the proposed arrangement of which is shown in figure 2.

The superficie ratio between sewage lagoon and each purification pond of a system of six in this case is approximately 8:1. However the exact proportion should be determined in the future by experience and research.

PROGRAM OF STUDIES

Determination of Water Quality

The water quality of the sewage lagoon will be studied according to the Standard Methods for Examination of Water and Waste Water edited by American Public Health Association, 1971.

After the six ponds are built and filled with sewage water but before the stocking of fishes, determination of water quality should be made (of both the lagoon and all the ponds) at least once a month according to the following criteria:

> Conductivity, total chlorine, detergent, ammonian nitrogen, nitrite and nitrate nitrogen, PO_4, SiO_2, Cu, Zn, CO_2, DO, COD, BOD, alkalinity, hardness, acidity, turbidity, temperature in air and water, etc.

Water samples will be taken at selected spots as indicated in figure 2. A 2-liter sampling cylinder will be made for this purpose.

Study of Fishes

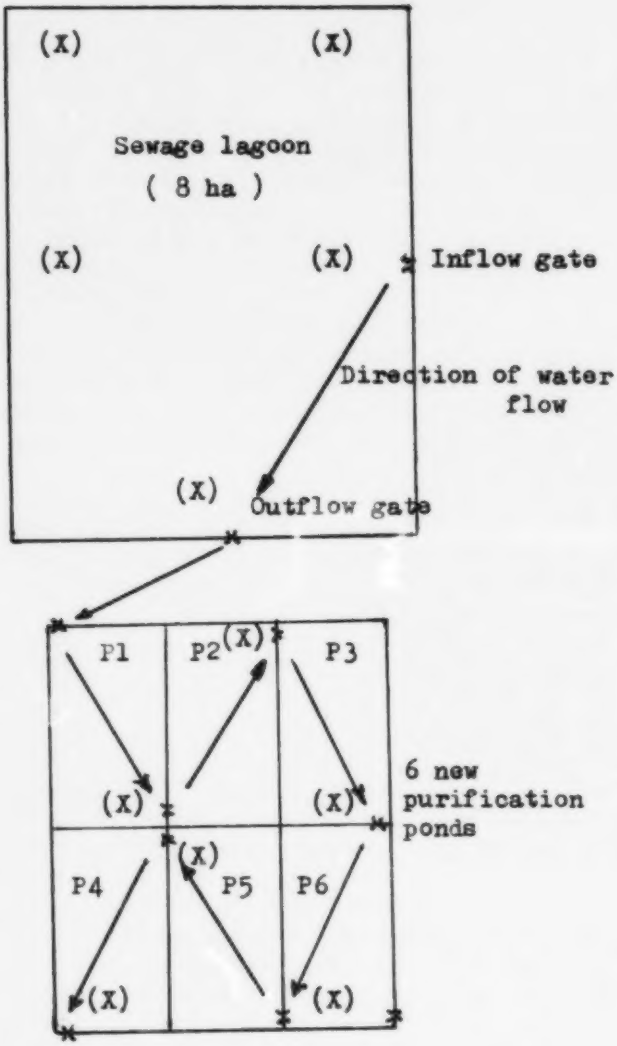
(X)
(X)
Sewage lagoon
(8 ha)
(X)
(X)
Inflow gate
Direction of water flow
(X)
Outflow gate
P1
P2
(X)
P3
(X)
(X)
6 new purification ponds
(X)
P4
P5
P6
(X)
(X)

Figure 2 - A sketch showing the existing sewage lagoon (above) and the six new purification ponds (below). (X) indicates the selected spot for water quality and plankton sampling.

Observation will be made on the daily movement of the fishes especially in relation to oxygen contents and their growth rates. As all the fishes stocked except the common carp are unable to spawn in captivity, popupulation study will be a simple matter. Even the common carp because of the depth of water and polluted conditions, may not be able to reproduce as well. Fish samples will be collected with nets from the lagoon and purification ponds once every three months during the period from April to October for weight and length measurements. In each sampling it is expected to obtain 20 to 50 silver amur, 5 white amur, 5 bighead, 5 black carp and possibly 3 common carp for examination.

Plankton and Benthos

The same water from samples collected for water quality determination will be used also for plankton study. Phytoplankton and zooplankton concentration, identification and number of each kind per liter will be determined according to limnological methods. Benthic biomass study will follow the method described in Hallock and Ziebell (1970).

Bacteria

Attempt may be made to determine whether coliforms are present at the outlet of pond 6.

Use of Aquatic Plants for Purification

Aquatic plants are to be grown in ponds P5 and P6 for the purpose of testing how effective phosphate and other nutrients left over would be absorbed by them. The water quality examinations at the inflows and outflows of the two ponds will be compared to determine this effect.

SELECTION OF FISHES FOR STOCKING

The fishes as described below all possess the characters that would meet the biological requirement for sewage wate water purification, but the most important common merit to them all is their tolerance to low dissolved oxygen content; they all thrive fairly well in a DO of 2 to 3 ppm and would grow well in 4 to 5 ppm at water temperature about 28°C; they can even survive for sometime below 1 ppm.

Silver amur - This silver fish is a very efficient phytoplankton feeder, fast growing to attain 5 to 10 kg in a few years. It lives in the upper layer of water, fast swimming, agile, active and always jumping out of water when disturbed; large fish can stand lower DO than small ones; a warm-water fish but tolerates temperature below 4°C. Because of the special spongy structure of its gill-rakers it can filter out phytoplankton less than 20 microns for food.

Bighead - A slow moving fish but fast growing, attaining large size up to 10 or 15 kg in a few years; a 10-pounder of three years old is not uncommon. The fish has a very large head, hence its name, and a wide mouth, living in the upper layer of water, gaping all the time to suck in phytoplankton, zooplankton and detritus for food. A warm-water fish ~~yey~~ tolerant to water temperature below 4°C; it survives under ice.

Black carp - A very large fish found in lakes and rivers of China up to 50 kg but in captivity it usually grows to some 10 kg in a few years. It prefers to live close to the bottom of deep water so that it can pick up snails, mussels and crustaceans for food.

White amur or grass carp - This fish is well known for its remarkable ability to consume satiably aquatic weeds, filamentous algae, land grass and leaves of many land plants. It roams every where in search of food; a hungry fish will take mixed feedstuff, meat, pieces of cloth or shoe leather when they are thrown into the pond, and yet it prefers fresh, tender vegetable food whenever they are available. It attains some 5 kg in three or four years on vegetable food alone, tolerant to water temperature below 4°C.

Common carp - A fish of longivity, perhaps a unique character among all fishes known; a color carp was recorded in Japan to live 215 years in a monastery. It inhabits most of the time near the bottom, sucking up worms and detritus mixed with mud. Immediately following the sucking the mud is ejected and the worms and digestible part of the detritus are retained and swallowed. As a result the bottom soil is thus disturbed and activated.

FISH STOCKING DESIGNS FOR EXPERIMENT

The design is based roughly on the estimates of phytoplankton production and the eating capacity of the plankton feeders. There will be designs of different fish communities for any particular sewage lagoon system.

When the six purification ponds each about one ha in superficie are built we would have a system of seven ponds including the sewage lagoon. According to the figures given by Tamiya (1957) and Ryther et al (1972) the sewage lagoon at Lonoke of 8 ha is capable , as estimated, to produce 1280 tons of phytoplankton a year, about 128 tons of which might be simultaneously converted to zooplankton. However to simplify calculation we may assume that the lagoon would produce a total of 1300 tons of plankton in all ayear or 6.5 tons a day if we count only 200 days a year as the active production period. Theoretically if all the plankton are to be removed, 13,000 to 32,500 kg of plankton-feeding fish should exist all the time in the lagoon and they would consume plankton 20 to 50% of their body weight.

Evidently it is neither possible nor desirable to deplete the lagoon of all the phytoplankton by stocking too many fish at one time, for a considerable quantity of phytoplankton must be left to do away with the noxious gases, excessive minerals and organic substances and to provide dissolved oxygen and CO_2 and consequently proper pH for the balance of life in the lagoon. For this reason it is proposed to stock the lagoon with a minimum rate of fish to feed on the plankton or empirically a rate of 1000 kg/ha or 8000 kg of fish for the whole lagoon of 8 ha, provided that the DO is sufficient for the fish to thrive. However, wherever the sewage lagoon is found to be insufficent in DO, no fish should be introduced and the effluent that flows into the purification ponds should be diluted with freshwater to improve the conditions for plankton growth and fish life. Anyhow at this rate of stocking, the silver amur, bighead and white amur of about 250 g would grow to one kg within a year which would be sufficient to consume enough plankton so as to prevent a turnover in the hot summer. As the fish grow and the lagoon is found overcrowded with fish the big ones should be removed and small ones introduced to replaced them.

A test fish stocking system of the lagoon and six ponds is suggested here as shown in table 1 for reference.

This table is given only to indicate a possible system of fish stocking . Modifications will have to be made when the waters of the lagoon and ponds are analised with attention being paid particularly to DO, NH_3 and other poisonous substances. As the primary sewage waste water is contimuously flowing into the lagoon and out from it carrying plankton at the rate of some 2.5% of the holding capacity of the lagoon and 10 to 20% of that of the purification ponds from one to another, the phytoplankton concentration must be accordingly reduced and therefore the fish stocking rate should be proportionately lowered.

ADDITIONAL MECHANICAL DEVICES IF NECESSARY

It is hoped that by this process of biological purification the effluent coming out of Pond 6 would be close enough to the EPA standards which stipulate as 30 ppm of BOD, 30 ppm of COD, 30 ppm of suspended solids, 200 coliforms per ml and a pH reaction of 9. However in view of the fact that after a considerably long period the accumulation of solids from the domestic sewage in the lagoon bottom will be too much for satisfactory functioning of the biological control system and the plankton and suspended solids may become higher than the standards, it may be necessary to consider, first of all, the installation of a sedimentation system with a fermentation tank (activated sludge method) to remove the solids before the city sewage is pumped into the lagoon. This is one part of the common installations in many mechanical engineering sewage treatment plants.

Table 1. A proposed stocking rate for the Lonoke sewage pond system

Lagoon and ponds	Area (ha)	Quantity of each species to be introduced						Total
		Silver amur	Bighead	Black carp	White amur	Common carp	Aquatic plants	
Sewage lagoon	8	6,000	1,000	400	200	200	--	7,800
P1	1	3,500	500	50	50	100	--	4,200
P2	1	2,500	500	50	50	50	--	3,150
P3	1	1,500	500	50	50	50	--	2,150
P4	1	1,000	300	50	50	50	--	1,450
P5	1	1,000	200	50	--	--	Selected plants	1,250
P6	1	500	100	50	--	--		650
Grand total	14	16,000	3,100	700	400	450		20,650

PROSPECTS OF FUTURE DEVELOPMENT

According to the records on municipal sewage system of Arkansas kept in the Department of Pollution Control and Ecology, State of Arkansas, January, 1972, there were 622,000 people who used "oxidation ponds" or lagoon for sewage treatment which cover a total of some 3,110 acres or 1,260 ha . If 945 ha more ponds are constructed to purify the effluents from the sewage logoons as described in the above proposed biological control project the total area will be increased to 2,205 ha. Country-wide statistics on sewage lagoons are not available at the moment, but should similar design be extended to the whole country at the same rate of one-third of the population or 70 million out of

210 million people, a total of sewage-aquaculture area of 247,350 ha would be required. In this aquaculture system primarily for the purification of sewage waste waters, if fish are stocked at the rate as shown in table 1, it would be possible to harvest a crop of 494,700 tons in total of marketable fish at the rate of 2000 kg/ha/year.

Objections may be raised against the allocation of land for the construction of the biological purification system. Some may think that land is too valuable for such a project and one or two hectares for every 300 people is excessive. But contradictions exist between clean air and water and the limited space of land. The urgency for the resolution of such contradictions allows us no alternative but to adopt a system which would be most economical and fit for maintaining the harmony and balance of natural environment for human life. It is therefore logical to argue that if clean air and water are desired sufficient land space must be provided for nature to work. Moreover consideration must be given to the high cost of establishing and maintaining a compact chemical and mechanical engineering plant against that of an economical biological control one. In the matter of environment protection principle, space is a basic factor for the maintenance of clean air and water, for unless sufficient space is allowed for nature to work in oxidation, reduction, sun energy activating photosynthesis and bacterial decomposition, all the wastes produced by men and animals cannot be recycled back to their original harmless components. For this reason it is not hard for us to realize that in city and rural community plannings, adequate land space and open air must be made available and amply reserved for parks, gardens, forest, recreation fields, roads, houses, hospitals, schools, sewage waste water disposal, factories and agriculture fields. When the fact that such planning is closely linked to public health and environment protection is kept in mind, one would agree that the provision of one or two hectares of land for the treatment of domestic sewage of every 300 people is nothing but a matter of absolute necessity.

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THE HARVESTING OF ALGAE AS A FOOD SOURCE FROM WASTEWATER USING NATURAL AND INDUCED FLOCCULATION TECHNIQUES

by

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INTRODUCTION

The autotrophic nature of algae has recently led to increased speculation as to the usefulness of these microorganisms in various nutrient removal schemes applicable to effluents from existing wastewater treatment plants. Since algae characteristically utilize inorganic compounds in the production of protoplasmic material, several tertiary treatment processes have been developed which focus upon the "stripping" of basic nutrients from solutions via normal algal metabolism. Whereas algae represent a concentrated mass of protein, fats, and carbohydrates, recent interest has also been expanded to include algal cultivation for possible commercial food usages. Therefore, a nutrient removal process which utilizes algae metabolism for "stripping" purposes represents a means of processing unwanted community wastes while economically, continuously, and quickly providing a relatively pure potential food product.

One of the serious drawbacks in any nutrient removal system involving algae is the separation of these microorganisms from the corresponding liquid phase. Regardless if one is considering nuisance conditions in surface waters, nutrient "stripping" processes, or the commercial production of algae for food supplies, feasible methods of harvesting algae in all cases becomes of utmost importance.

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Among the various mass-liquid separation processes that have been investigated with regard to their potential for algae harvesting include: centrifugation, filtration, flotation, ion-exchange, sedimentation, straining, chemical treatment, and bioflocculation. While the majority of these methods are quite costly, properly managed flocculation techniques may offer a feasible, as well as economical, solution to algal-liquid separation. The purpose of this investigation was therefore focused on describing the feasibility of harvesting algae as a food source from wastewater treatment systems using natural and induced flocculation techniques.

LITERATURE REVIEW

Unlike bacterial aggregation, very little published research information is available pertaining to algal agglutination. Initiating a discussion of algal flocculation mechanisms necessitates an understanding of algal surface charge.

Most algal cells (with the exception of certain filamentous forms) fall in the size range of 5 to 50 µm. Although algae are larger than true colloids (1 to 100 nm), they nevertheless possess many surface properties similar to true colloids. Discrete algal cells are known to form stable microbial suspensions, possess a chemically reactive cellular surface, and possess a net negative surface charge (1) (2) due to the ionization of functional ionogenic groups. Algae may be considered as hydrophilic bio-colloids since algal suspension stability depends not only on the forces interacting between the particles themselves, but also on the forces interacting between the particles and the water.

Algal Bioflocculation Literature

Due to the limited amount of research conducted on algal bioflocculation, documentation in the published literature of actual floc formation is scarce. The agglutination of *Stigleoclonium stagnatile* into settleable flocs resembling aerated activated sludge was observed by Bogan, Albertson, *et al* (3) during a 1960 investigation of phosphorus removal by algae. It was postulated that the enclosure of the cell by a broad gelatinous sheath (4), usually characteristic of these filamentous algae, may have promoted entanglement of filamentous algal appendages with consequent enhancement of bioflocculation.

A review of algal harvesting processes by Golueke and Oswald (5), in 1965, resulted in the attainment of bioflocculation under specific environmental conditions. The necessary requirements were an actively photosynthesizing shallow culture, a relatively warm day, and sunlight. However, as in the previous investigation, no hypothesis for the algal bioflocculation mechanism was proposed.

In 1967, Schuessler (6) proposed an algal bioflocculation model in which naturally produced polysaccharide polymers would bridge between discrete algal cells. In his studies, bioflocculation potential was correlated with the algal growth phase - maximum algal removals being observed during declining growth. Schuessler pointed out that declining growth was a period of increased polysaccharide production and excretion, thereby affording optimal bridging between linearly extended polysaccharide polymers and algal cells with resultant bioflocculation. Experimental results also noted that high algal removals due to bioflocculation were observed at both low and high pH levels. For the low pH band from 1.0 to 4.0, in which algae are in close proximity to their isoelectric point, effective bioflocculation was reported with best results at pH 3.0. For the high pH band from pH 11.0 to 13.0, the algal removal efficiency was greater than that observed in the low pH region and was attributed to the formation of hydroxyl and carbonate precipitates that served to enmesh the algae with resulting removal.

In 1969, Tenney, Echelberger, et al (7) postulated a bioflocculation model in which high molecular weight exocellular metabolites, which are of sufficient length, bridge between discrete algal cells, thereby initiating the bioflocculation process. They contended that the microbially produced polymers initially might enhance floc formation, while in later growth phases the accumulation of this extracellular material could be acting as a protective colloid.

While investigating further the effects of algal exocellular polymer production on the bioflocculation process, Pavoni (8) reported, in 1971, that algal cell aggregation appeared to be governed by the physiological state of the microorganisms. Observations indicated that floc formation was restricted to the declining growth phase. In this study, direct correlations were developed relating exocellular polymer production and algal agglutination. Pavoni, therefore, postulated surface coverage phenomenon as the mechanism of algal bioflocculation, in which case surface potential reduction was not necessarily a prerequisite for agglutination.

The excretion or exposure of natural polymers on the algal surface during photosynthesis is widely documented in the literature with the implication by several investigators (9) (10) that the mechanism of algal bioflocculation is the bridging function of naturally produced polymeric species. It, therefore, seems appropriate to discuss the specific types and quantities of these algal exocellular materials referred to in the literature.

In 1849, Kutzing's original description of Navicula pelliculosa (Synedra minutissima and pelliculosa), according to German (11), made reference to an algal gelatinous capsule - "Individua in gelatina membranacea nidulantia" - which has given the organism its present specific name.

In 1851, reports by Magin (12) indicated that through the use of dyes Bailey found an external envelope of complex carbohydrates to exist around the cell walls of diatoms. Liebisch (13) showed these exocellular secretions to have the same chemical nature as pectic compounds in 1929.

Various fresh and salt water algal species were observed to secrete organic materials by Krough (14) and Aleev (15) during the 1930's. In 1950, Locker (16) noted the presence of slime on the surface of ponds containing an abundance of Navicula pelliculosa. She noted the capsule of this species, when cultured in the laboratory, sustained a positive carbohydrate stain.

Microscopic examinations of Anabaena, an algal species from which Bishop, Adams, et al (17) isolated an exocellular polysaccharide, showed many of the filaments were enclosed in a mucilaginous substance which was readily sloughed off into the medium. This report of nitrogenous material secretion by blue-green algae was supported in 1952 by Fogg (18) who found polypeptide and amide secretion by young cultures of Anabaena, a species of blue-green algae. That same year Hough, Jones, et al (19) observed amylopectin-like polymers in Oscillatoria while the species Nostoc yielded a mucilaginous, complex, acidic polysaccharide composed of a minimum of six distinguishable monosaccharides.

In 1954, Bailey and Neish (20) found that polysaccharide reserves, composed of amylose and amylopectin, constituted up to 20 percent of the dry weight in some algal cultures.

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Secretions of peptides, amides, and amino-nitrogen were observed by Fogg and Westlake (21) the next year, and their report included emphatic remarks as to the importance of such secretions to the ecology of fresh waters.

Lewis and Rakestraw (22) found carbohydrates in seawater in quantities of from 0.1 to 0.4 mg/l and attributed it to the secretion products of planktonic algae. While Lewin (23) found no evident capsule around actively dividing *Navicula pelliculosa* cells, he did observe the formation of a gelatinous capsule, composed of glucuronic acid residues, following the cessation of cell reproduction.

Polysaccharide and organic acid secretion by green algae was reported by Tolbert and Zill (24) and Allen (25) in 1956. Extending this specific research with unicellular and colonial green algae, Lewin (26) observed that all *Chlamydomonas* species studied liberated some soluble polysaccharides into the growth medium. This material was found to constitute 25 percent of the total organic matter produced by the cells of *Chlamydomonas mexicana*. In all but one species, galactose and arabinose were the main components of the polysaccharides. Lewin also postulated other possible algal metabolites including free organic acids and polypeptides.

Collier (27) demonstrated carbohydrate production in bacteria-free cultures of *Prorocentrum*, in 1958, while discussing the importance of naturally produced organic compounds in surface waters. Krauss (28) also reported finding an exocellular polysaccharide material excreted by the genus *Oscillatoria*. The same year Fogg and Boalch (29) noted extracellular polysaccharide and nitrogenous compounds in *Ectocarpus confervoides* and concluded that both types of exocellular products are liberated from healthy cells.

Carbohydrate accumulation, occurring initially during declining growth and continuing into the endogenous growth phase, was observed by Guillard and Wangersky (30). They stated that cell demise liberated comparatively large amounts of carbohydrate which could account for the presence of this material during the active growth phase. Guillard and Wangersky, noting that algal photosynthetic reserves amass during stationary growth under circumstances unfavorable for the utilization of external carbohydrates as an energy source, also postulated that these reserve materials consist largely of other materials such as polypeptides and organic acids, which may also be present in the growth medium.

Merz, Zehnpfenning, et al (31) investigated species of Chlamydomonas, Chlorella, Chloracoccum, and Scenedesmus, in 1962, and noted some production of soluble exocellular organic matter by the majority of autotrophic algal species studies.

More recently, Moore and Tischer (32) reviewed the three classes of organic compounds known to be liberated by freshwater algae - organic acids, nitrogenous material such as polypeptides and free amino acids, and carbohydrate polymers. Their investigation was specifically concentrated on the exocellular polysaccharide content of eight species of green and blue-green algae. Results indicated total polysaccharide production in amounts equal to 10 to 40 percent of the algal dry weight.

In summary, a large variety of organic substances have been detected in the exocellular products of algae. These materials can be classified into three general categories: proteins, polysaccharides, and nucleic acids. With the structure of most of these substances being polymeric and with these materials possessing a net negative charge in neutral pH ranges, the exocellular products may be considered to respond as naturally produced anionic polyelectrolytes.

There is, however, a scarcity of information regarding the role of these exocellular products in the algal agglutination mechanism. Consequently, any refinement of the algal agglutination process would entail investigations into the composition of the exocellular polymers, the mode of polymer release from the cell, and the role of cell surface potential.

Chemical Flocculation of Algae

The feasibility of removing algae from water and wastewater by chemical flocculation techniques has been studied by several investigators. The available literature, although limited, indicates the apparent feasibility of utilizing this technique for algal removal. In 1958, Cohen, Rourke, et al (33) conducted studies involving the agglomeration of Chlorella cells with synthetic organic polyelectrolytes. Results indicated that good flocculation was achieved while using a cationic polyelectrolyte, whereas no flocculation was observed with the anionic polymer.

Golueke, Oswald, et al (34) indicated excellent aggregation of Chlorella and Scenedesmus with cationic polyelectrolytes in 1964. In these studies the requisite polyelectrolyte dose was insensitive to pH in the range of 5.0 to 10.4; however, increasing concentrations of dispersed algal cells increased the requisite polyelectrolyte dose proportionately. Algal flocculation with anionic or nonionic polyelectrolytes was not investigated.

More recently field scale pilot plant studies by the Dow Chemical Company have served to demonstrate further the feasibility of utilizing cationic polyelectrolytes as algal flocculants.

In 1969, Tenney, Echelberger, et al (7) investigated the feasibility of removing algae from water and wastewater with synthetic organic polyelectrolytes. Representative cationic, anionic, and nonionic synthetic organic polyelectrolytes were used as flocculants. Under their experimental conditions, chemically induced algal flocculation occurred with the addition of cationic polyelectrolytes, but not with anionic or nonionic polymers, although attachment of all polyelectrolyte species to the algal surface was shown. It should be noted, however, that anionic and nonionic polymeric flocculation was only studied at pH 7.0.

Obviously, very little information is available which begins to delineate the parameters involved in the chemical flocculation of algae using anionic or nonionic polyelectrolytes. It would appear that a basic need exists, not only for the development of quantitative data regarding the effectiveness of selected anionic and nonionic polyelectrolytes as algal flocculants, but also for the evaluation of the basic mechanism of algal-flocculation interaction and the associated parameters affecting this interaction. Also, since synthetic organic anionic and nonionic polyelectrolytes most probably closely resemble natural exocellular algal polymers, a correlation may exist between synthetic polymer-algal interactions and natural polymer-algal interactions.

EXPERIMENTAL PROCEDURE

The general experimental approach employed throughout this investigation was to select scientifically established techniques that would not only allow proper evaluation of basic theoretical parameters, but also would be flexible enough to thoroughly support reasonable variations in experimental protocol. Analytical procedures chosen were sufficiently sensitive and specific for the scope of this investigation but, nevertheless, were not overly sophisticated to the point of being nonapplicable to the wastewater treatment field.

Laboratory scale batch algal culturing reactors were used throughout this investigation. The initial algal inoculum was obtained from the inside wall of a secondary clarifier at the Hite Creek Wastewater Treatment Plant in Louisville, Kentucky. A typical algal culturing system consisted of a 40-liter glass jar, a stone air diffuser which supplied both aeration and complete mixing, a large cooling fan, and a bank of flourescent lamps developed specifically to stimulate photosynthesis. This light source imparted a culture illumination of 400 foot-candles to the algal surface with an energy dominance in the blue and red regions of the visible light spectrum.

An initial algal concentration of approximately 10 to 20 mg/l was inoculated into 25 liters of a standard liquid algal medium (35), and daily pH adjustments were performed when necessary to insure a pH between 7.0 and 7.5. All evaporation losses were made up with distilled water each day previous to sample withdrawal. Culture temperature was maintained at 22°C±2°C.

Once an algal batch reactor was begun, the experimental procedure consisted of recording several parameters including:

1. algal mass (dry weight basis) in accordance with the procedures of Engelbrecht and McKinney (36).
2. culture flocculation as a function of either cake filtration time or percent transmission at 520 mμ.
3. algal cell surface charge using a Riddick Zeta Meter (37).
4. extraction of exocellular polymer for quantitative monitoring (dry weight basis) and fractional composition analysis.

Exocellular polymer extraction techniques utilizing ethanol were employed similar to the work of Ueda, et al (38). Three samples secured from batch reactors were centrifuged at 32,500 g for 15 minutes to affect shearing of polymeric material from the algal surface. The supernatent from this centrifugation was added to 95 percent ethyl alcohol so that the final ratio of volume was two parts ethanol to one part supernatent. The supernatent-ethanol mixture was refrigerated for 24 hours at 4°C after which time a white fibrous precipitate was observed. Quantitative monitoring of microbial polymer production was accomplished at this point by performing a membrane filter suspended solids test on two of the original three samples.

The white precipitate formed in the third sample was then separated from the supernatent-ethanol liquid phase by centrifuging at 32,500 g for 15 minutes and dissolved in distilled water. This polymer solution underwent qualitative colorimetric analysis for polysaccharide (39), protein (40), ribonucleic acid (RNA) (41), and deoxyribonucleic acid (DNA) (42).

Flocculation of algal dispersions with synthetic organic polyelectrolytes was performed utilizing standard jar testing procedures (7). Algal suspensions were secured by harvesting algae from batch cultures by means of centrifugation at 32,500 g for 15 minutes and resuspending the algal pellet in a 0.5 percent aqueous sodium chloride solution. This centrifugation procedure was repeated to insure proper contaminant removal. Organic flocculants used were produced by the Dow Chemical Company and included cationic polyelectrolytes (C-31 and C-32), anionic polyelectrolytes (A-22 and A-23), and nonionic polyelectrolytes (N-11, N-12, and N-17).

RESULTS AND DISCUSSION

Algal Bioflocculation Studies

Initially, laboratory batch fed algal cultures were nurtured to study the relationship between algal growth, culture turbidity, exocellular polymer production, exocellular polymer to algal mass ratio, exocellular polymer composition, and cell surface charge. If current polymer bridging models are to be expanded to explain algal bioflocculation, then it should be possible to correlate algal culture turbidity decrease with an increase in exocellular polymer production. Additionally, algal cell surface charge would be expected to remain constant throughout the entire growth cycle.

The first study was comprised of a batch fed heterogenous algal culture and was focused on duplicating and refining several correlations that had previously been shown to exist for algal bioflocculation phenomenon. A microscopic analysis of this culture was performed to ascertain the predominant algal species present in the medium. Chorella and a filamentous strain were observed in abundant numbers; however, a determination of which algal type predominated and the exact identification of the filamentous strain were not made.

The data from culture 1, as presented in Figures 1 and 2, clearly demonstrate several significant aspects of biological flocculation. Algal agglutination (culture turbidity decrease) was observed to occur only after the algae had entered endogenous growth stages (see Figures 1A and 1B). The dependence of the algal bioflocculation process on the physiological state of the algae agrees with previous investigations by Pavoni (8) and parallels results obtained for similar bacterial systems (43). Filtration time (see Figure 1C) was also observed to be a qualitative measure of algal bioflocculation in agreement with the work of LaMer and Healy (44). The filtration time in this culture was observed to reach a maximum prior to endogenous growth and decrease steadily from that point as bioflocculation phenomenon increased.

If current polymer bridging models are to be expanded to explain algal bioflocculation, it should be possible to correlate algal culture turbidity decrease with an increase in exocellular polymer production. As indicated in Figure 2A, the algal exocellular polymer concentration in culture 1 increased significantly during endogenous growth (a period of enhanced agglutination). The inference, as in bacterial systems, is that this exocellular polymer is the primary cause of bioflocculation during endogenous growth. Since the ratio of exocellular polymer to algal mass sharply increases during culture aggregation (see Figure 2B), the polymer bridging model of bioflocculation is further supported. Correspondingly, the increase in this ratio indicated that surface coverage phenomenon may have been responsible for increased agglutination.

Another important aspect of the bioflocculation process is the mode of polymer release from the algal cell. Algal aggregation was noted as being limited to endogenous growth with a concurrent increase in exocellular polymer concentration during the growth phase. Since restricted growth is a period of massive cell lysis, algal autolysis may have been the mechanism of polymer release.

Whereas culture 1 was investigated solely for the purpose

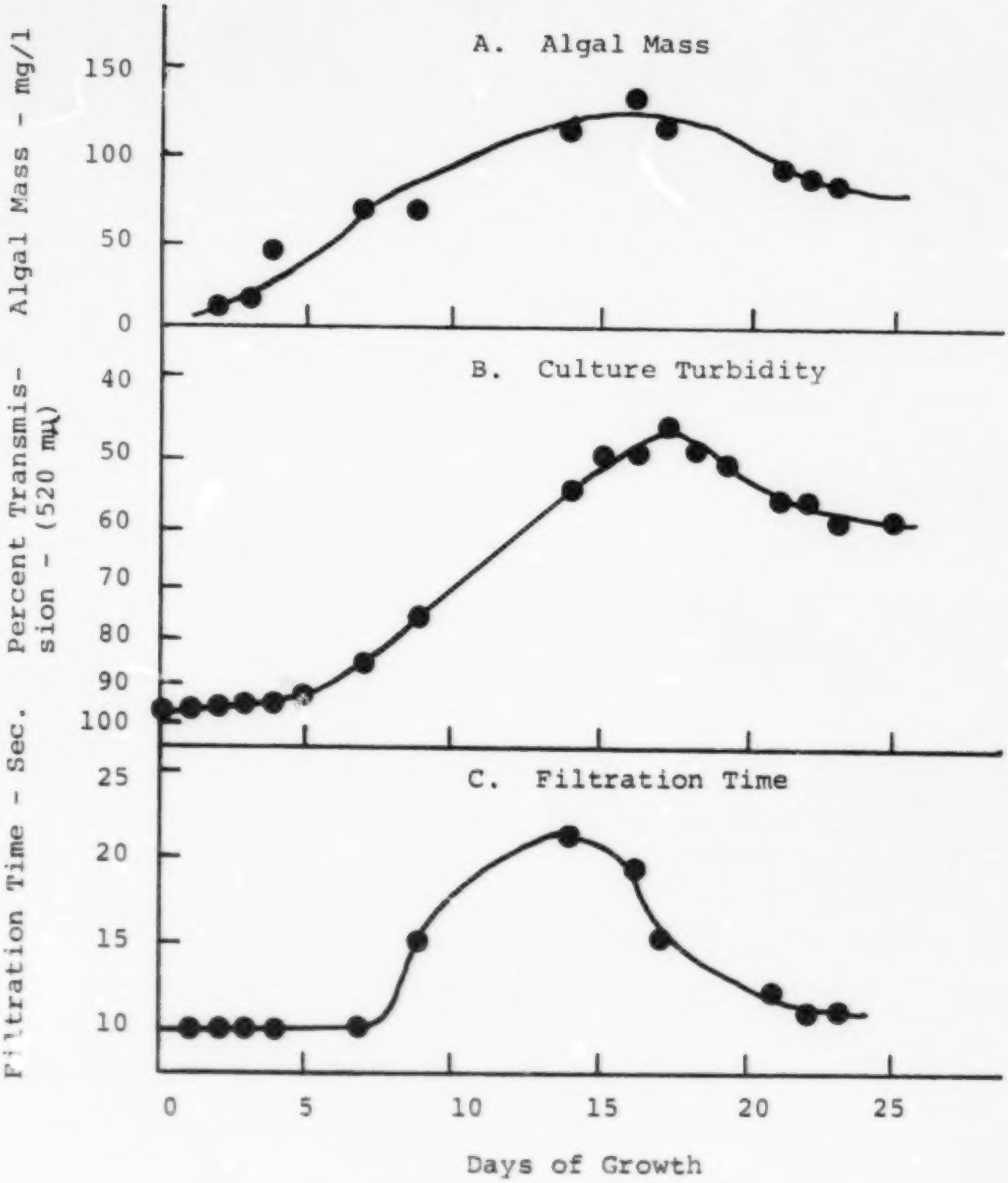


Figure 1 Culture 1 Data Depicting (A) Algal Growth, (B) Culture Turbidity, and (C) Filtration Time.

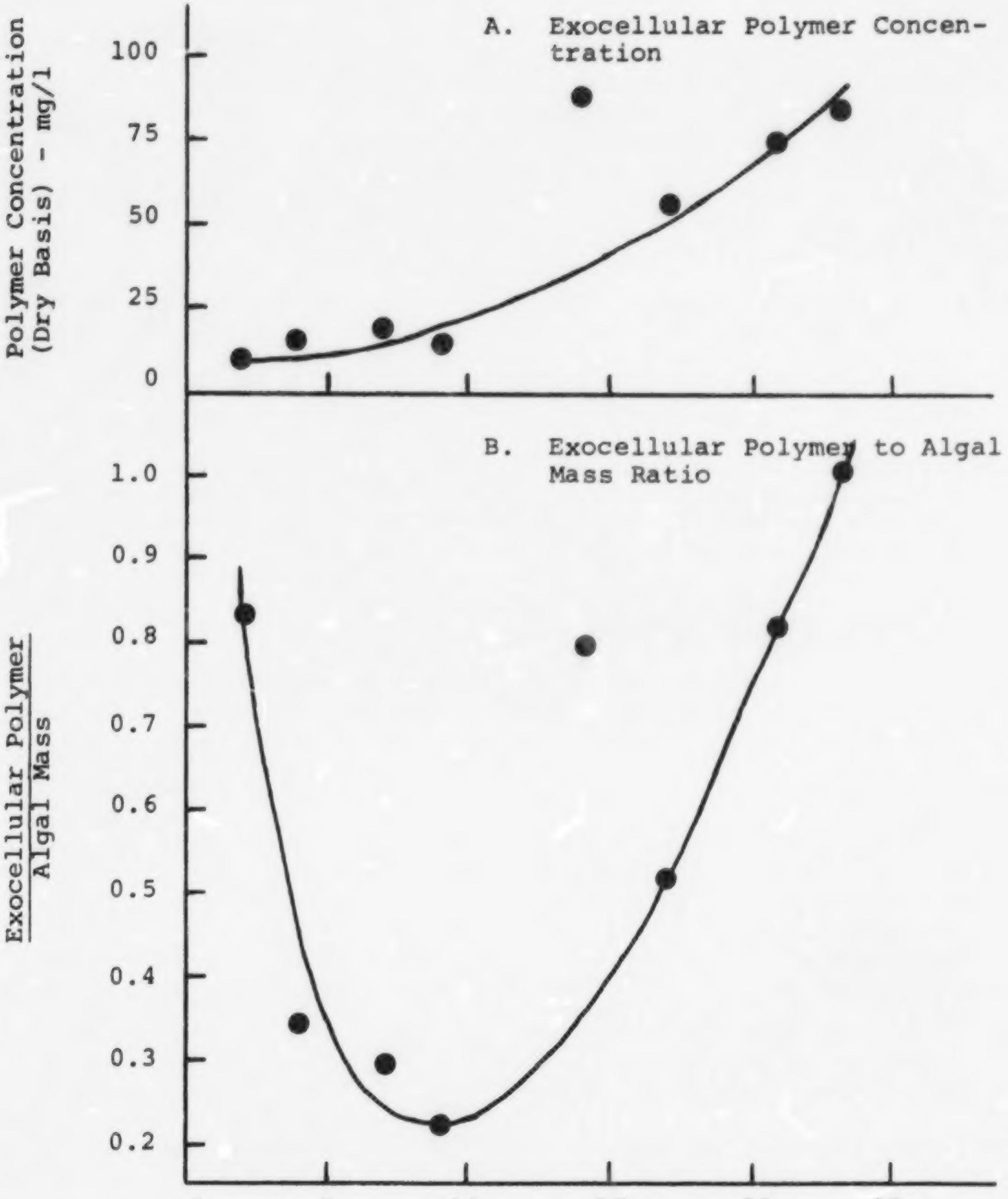


Figure 2 Culture 1 Data Depicting (A) Exocellular Polymer Production and (B) the Ratio of Exocellular Polymer to Algal Mass.

of refining the characteristics of previously observed phenomena, culture 2 was developed and monitored to determine the algal cell surface charge through the entire growth cycle and the fractional composition of extracted exocellular polymer. Knowledge of cell surface charge and exocellular polymer compositional makeup could significantly shed light on the understanding of the overall bioflocculation process.

A microscopic analysis of culture 2 revealed the presence of a dense population of *Chlorella* with only occasional sightings of filamentous strains. Therefore, *Chlorella* were determined as being the predominant species in this culture.

The results of culture 2 relevant to the refinement of previously observed bioflocculation phenomenon paralleled those of culture 1, i.e., a correlation was observed between the endogenous growth phase and enhanced aggregation (see Figures 3A and 3B). Filtration time was again observed to serve as a qualitative indicator of bioflocculation phenomenon (see Figure 4A).

If polymer bridging theories are to be expanded to explain algal bioflocculation, cell aggregation should be independent of surface potential reduction. The algal cell zeta potential was monitored in order to evaluate the algal surface charge during the complete growth cycle of culture 2. Figure 4B shows that no significant changes in zeta potential were observed during any algal growth phase. The stability of the cell surface charge indicated that surface potential reduction was not a prerequisite in the bioflocculation mechanism. Since a bioflocculation model based on some measure of charge neutralization would involve the formation of tightly compacted cell aggregates, the independence of surface charge and algal bioflocculation observed in this investigation added support to the polymer bridging theory in which discrete cells are loosely bound in a three-dimensional matrix.

Algal exocellular polymer produced in culture 2 was also extracted and weighed to develop polymer/algal mass ratios. Unlike results observed in culture 1, the polymer concentration in culture 2 remained virtually constant throughout all growth phases with only a minor increase detectable during endogenous growth (see Figure 5A). Although the exocellular polymer to algal mass ratio increased during endogenous growth, this increase appeared incremental when compared to that obtained in culture 1 (see Figure 5B).

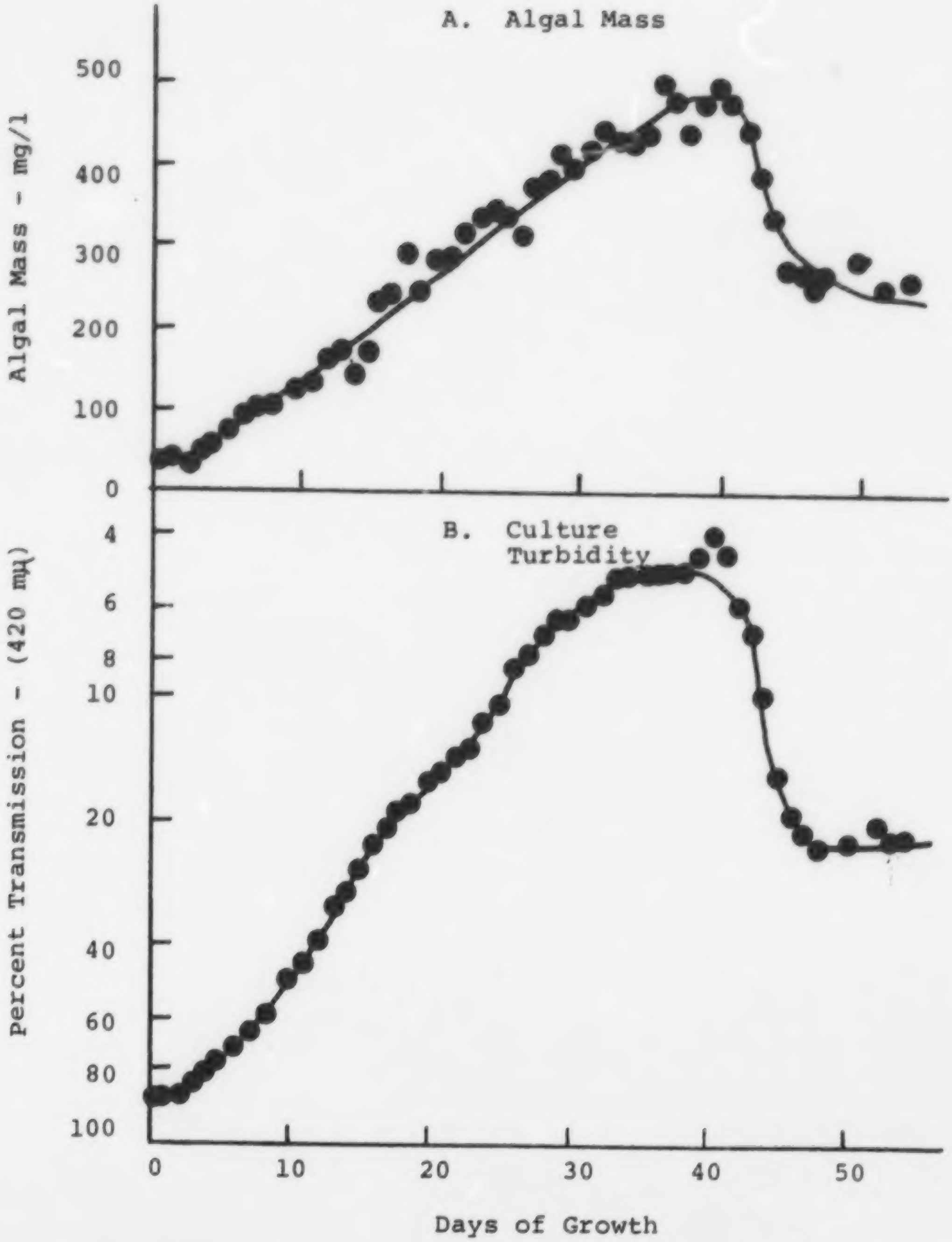


Figure 3 Culture 2 Data Depicting (A) Algal Growth and (B) Culture Turbidity.

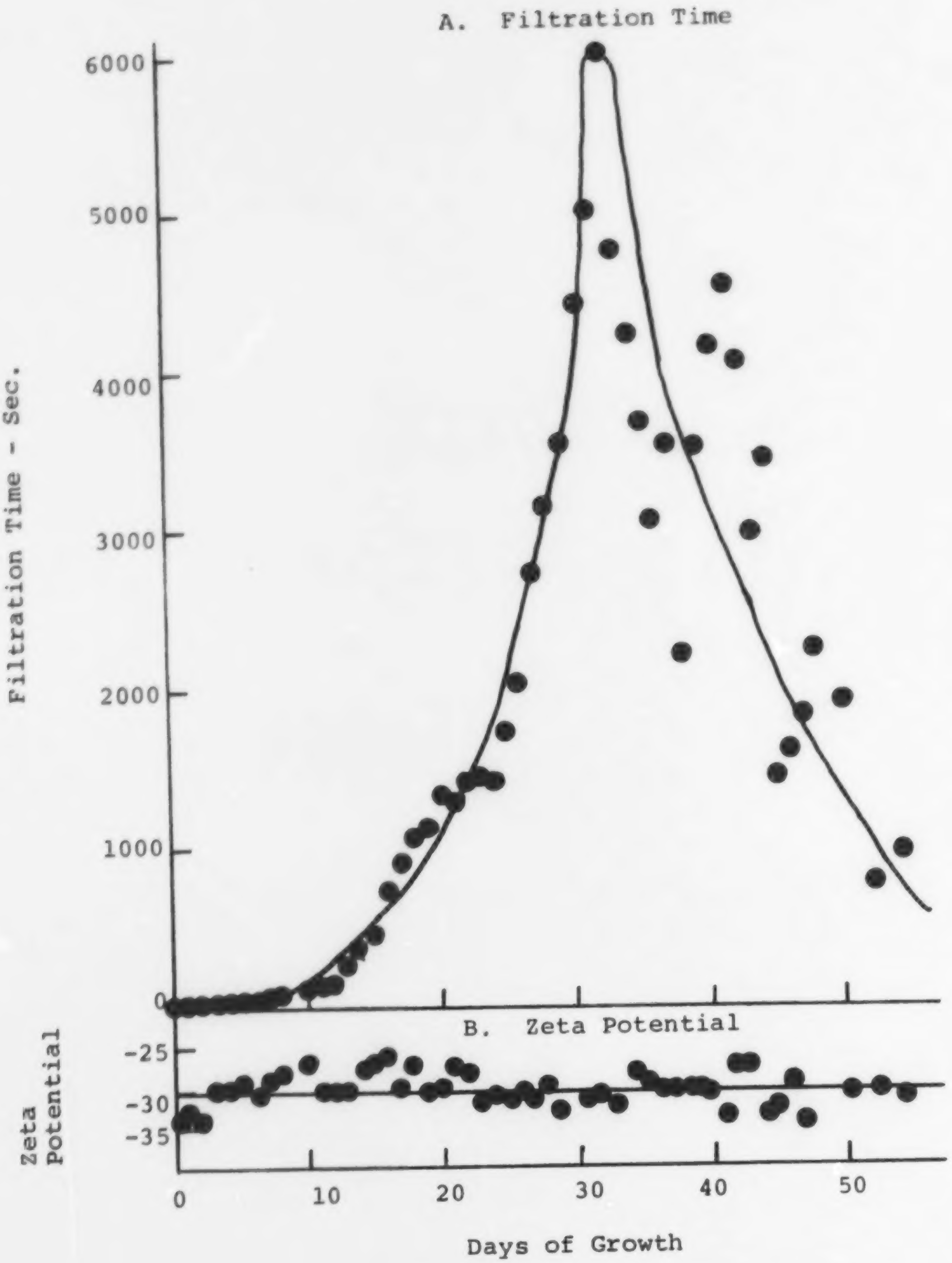


Figure 4 Culture 2 Data Depicting (A) Filtration Time and (B) Cell Surface Charge.

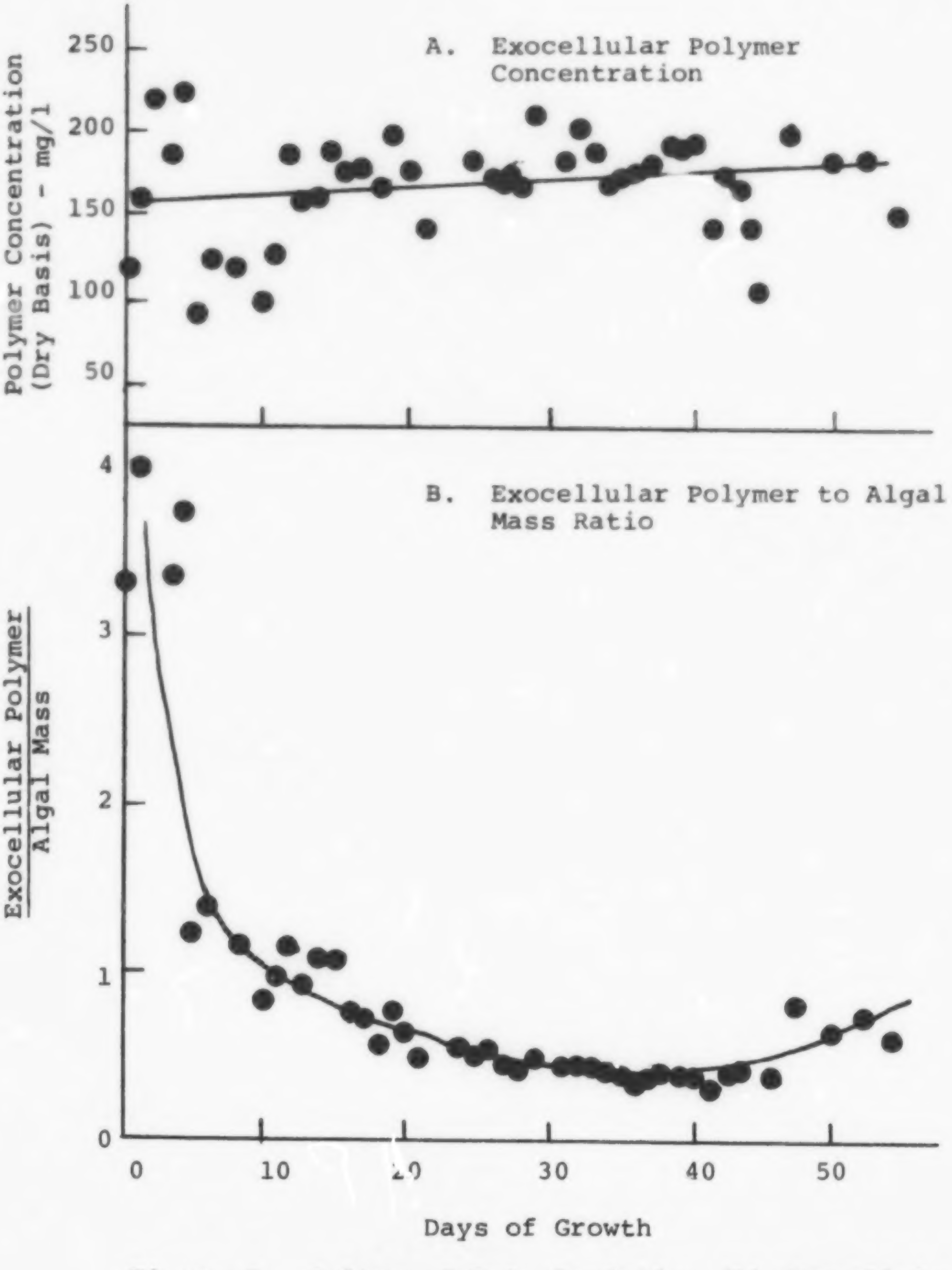


Figure 5 Culture 2 Data Depicting (A) Exocellular Polymer Production and (B) the Ratio of Exocellular Polymer to Algal Mass (from Weight Analysis).

It is believed, however, that the extracted polymer in culture 2 was contaminated with inorganic material. Friedman, et al (45), while isolating exocellular polymer from Zoogloea ramigera, observed the presence of interfering materials in the polymeric precipitate.

Consequently, another polymer production curve was developed for culture 2 by adding the concentrations of the individual components (polysaccharide, protein, RNA, DNA) found in the fractional composition tests. This curve, presented in Figure 6A, shows a more significant increase in exocellular polymer concentration during endogenous growth, and the ratio of exocellular polymer to algal mass also shows a marked increase during late growth (see Figure 6B).

In comparing the polymer production curves obtained from weighing the polymer and from the fractional composition analysis (refer to Figures 5A and 6A), a substantial difference in polymer values can be observed. This difference was postulated as arising from the precipitation of inorganic constituents which increased the polymer totals in the weighing procedure, but had no effect on the specific compositional analysis techniques. To determine whether or not inorganic interference was occurring, several observations were made of precipitated polymer. Following filtration of a polymer sample, examination of the membrane filter indicated the presence of not only long strain polymeric fibers, but also fine, granular particles. Consequently, a membrane filter containing the filtered precipitate was combusted in a muffle furnace at 600°C. Since the filter itself left no ash, the residue remaining represented inorganic contaminants present in the sample. It is important to note that a significant amount of residue was observed after combustion. Therefore, it can be assumed that inorganic precipitates were formed during the ethanol extraction in agreement with Friedman, et al (45).

Of major importance in a description of the algal bioflocculation process, as developed from the polymer bridging model, is the composition of the exocellular polymer. Samples of the polymer-ethanol mixture were centrifuged to separate the polymer from the liquid phase. The exocellular polymer was then added to 20 ml of distilled water and fractional composition analysis techniques were performed. The analysis was designed to categorize the polymeric components into the broad classifications of protein, RNA, DNA, and polysaccharide. Figures 7A, 8A, 9A, and 10A represent the concentrations of the major components of

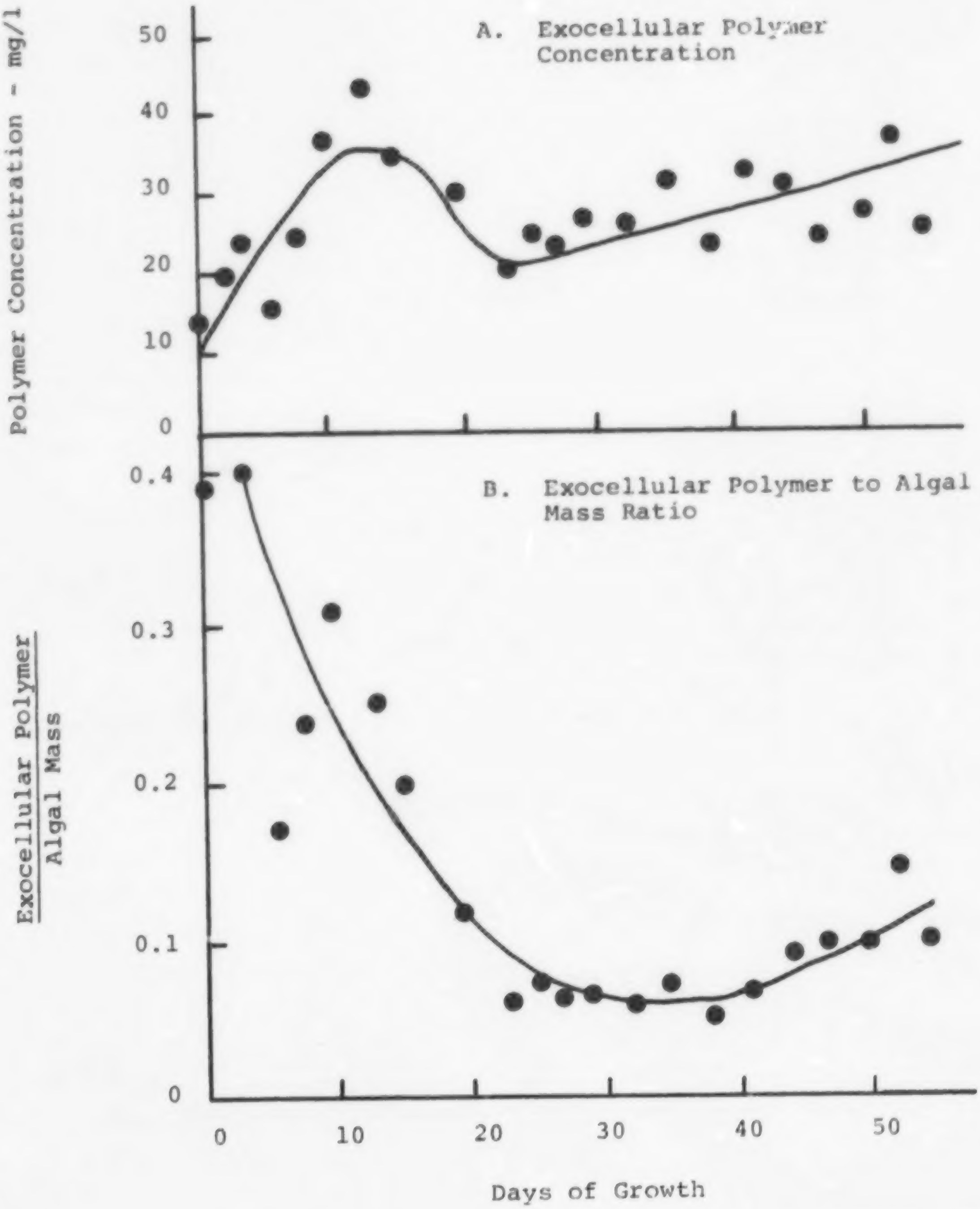


Figure 6 Culture 2 Data Depicting (A) Exocellular Polymer Production and (B) the Ratio of Exocellular Polymer to Algal Mass (from Fractional Composition Analysis).

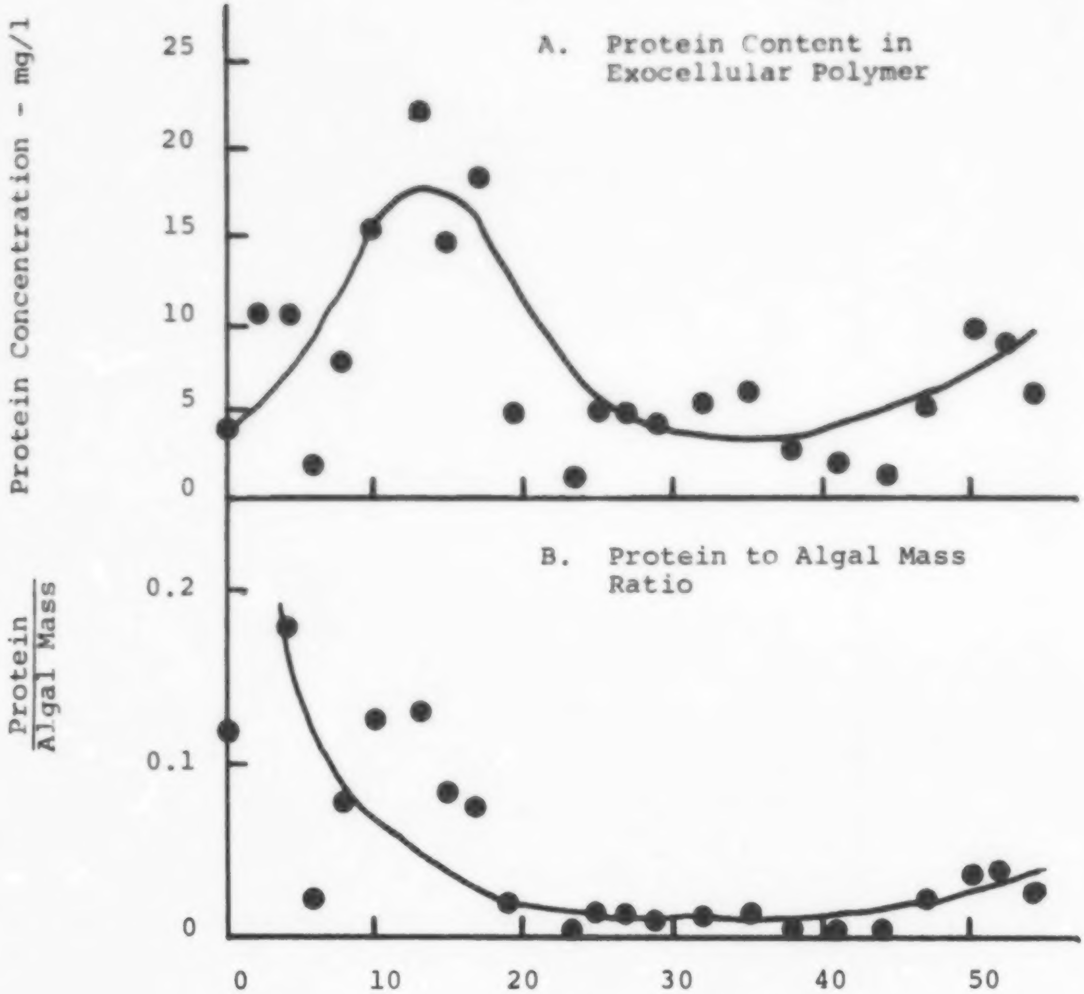


Figure 7 Culture 2 Data Depicting (A) the Protein Fraction of Exocellular Polymer and (B) the Ratio of Protein to Algal Mass.

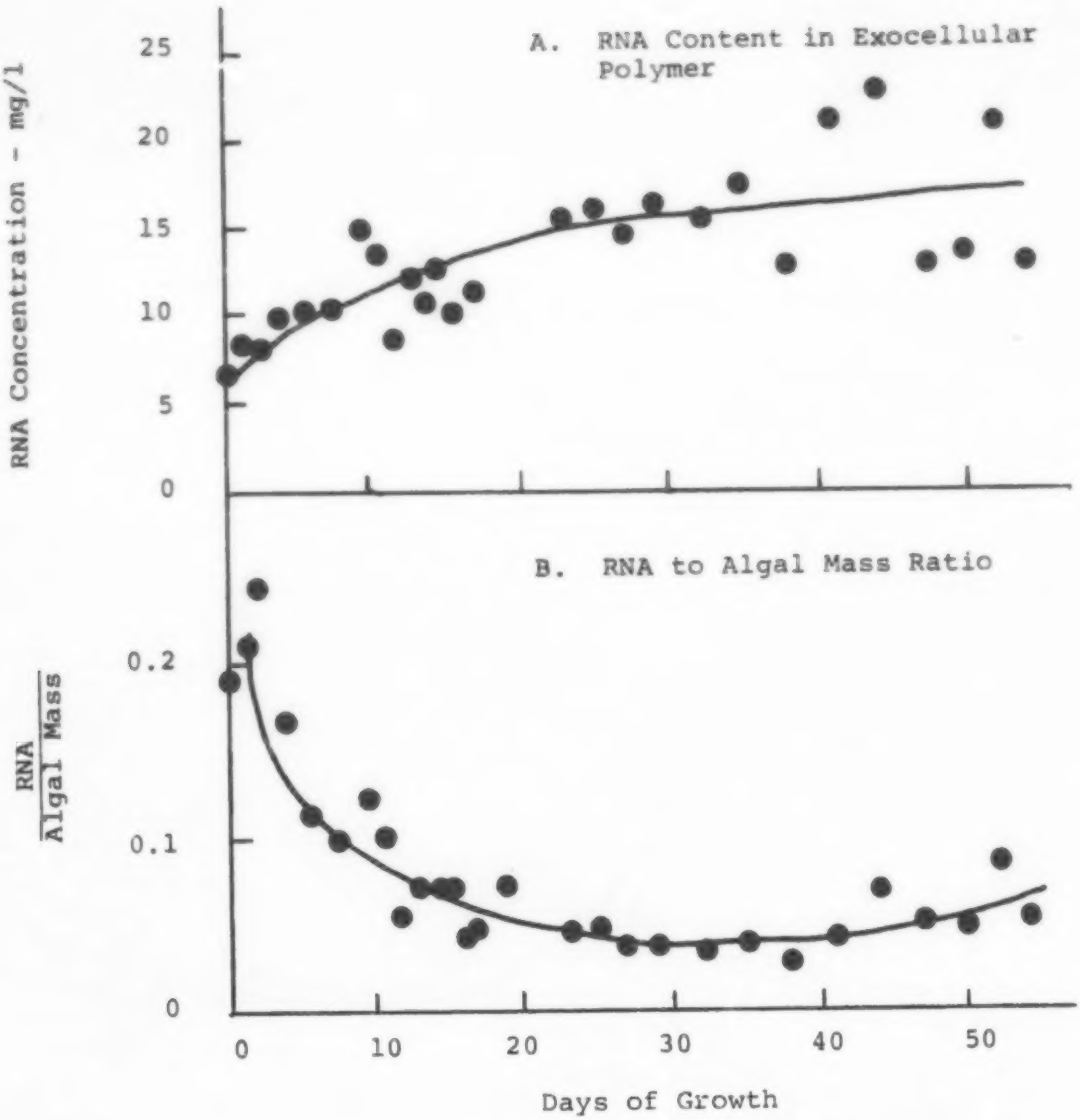


Figure 8 Culture 2 Data Depicting (A) the RNA Fraction of Exocellular Polymer and (B) the Ratio of RNA to Algal Mass.

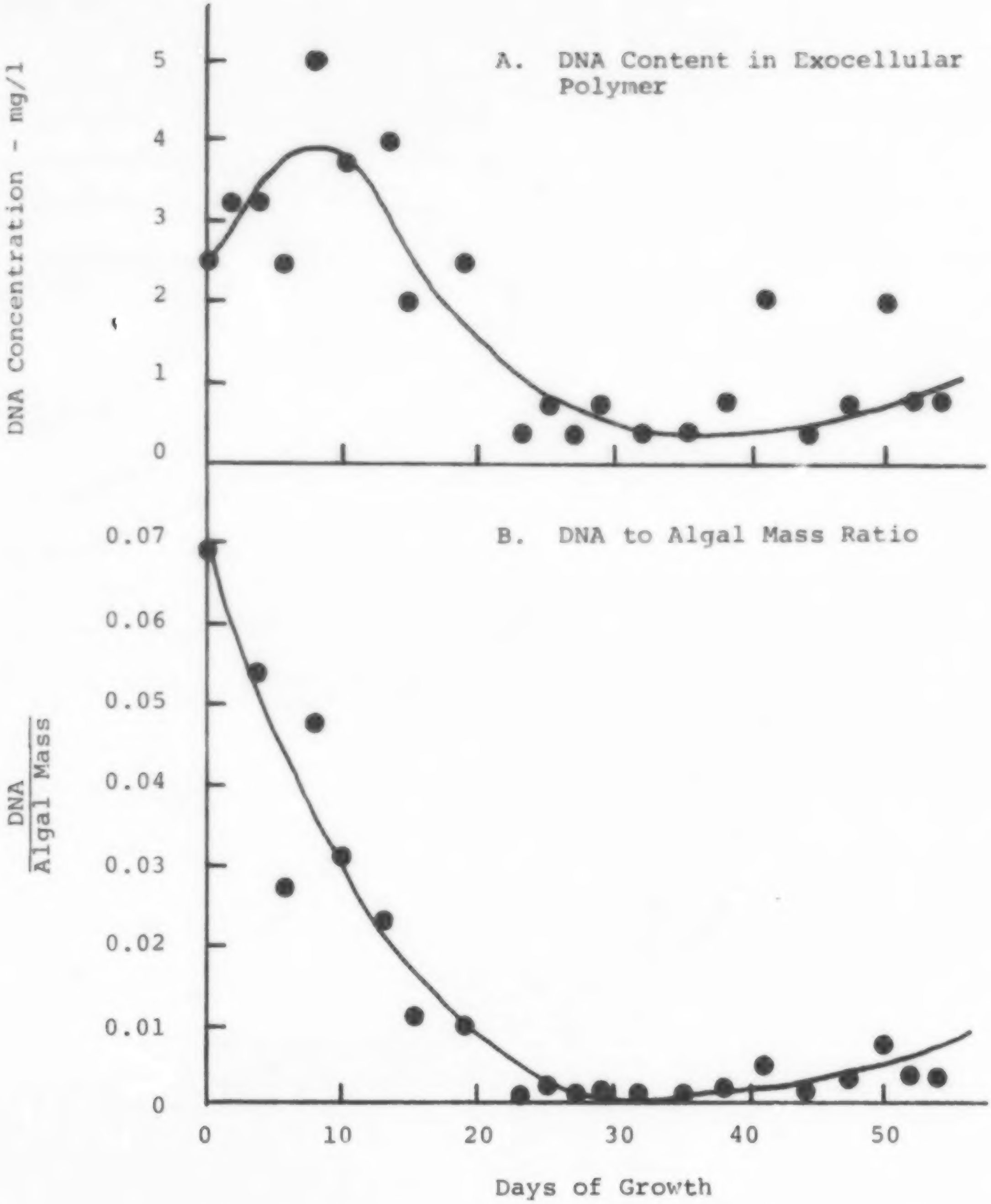


Figure 9 Culture 2 Data Depicting (A) the DNA Fraction of Exocellular Polymer and (B) the Ratio of DNA to Algal Mass.

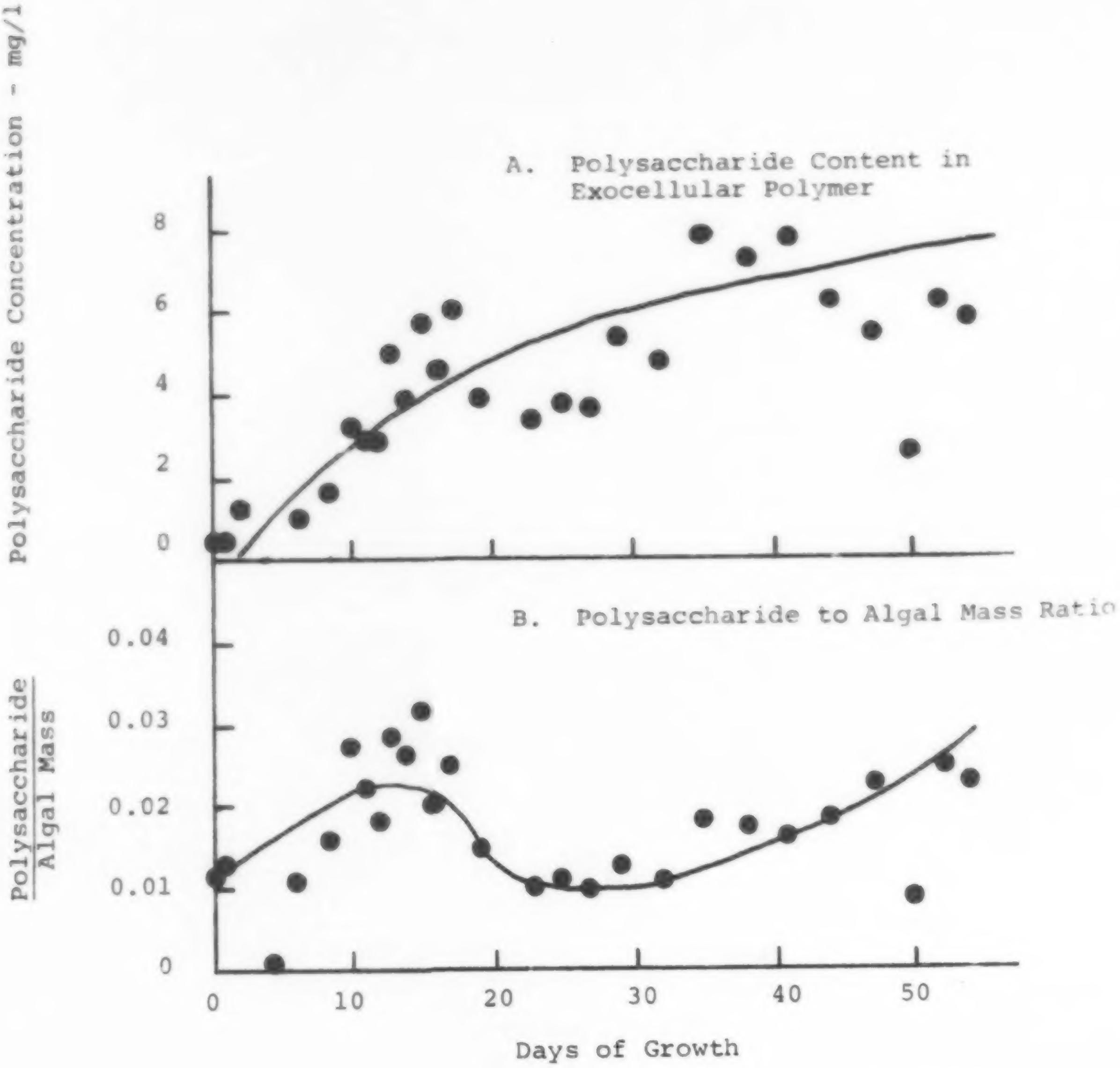


Figure 10 Culture 2 Data Depicting (A) the Polysaccharide Fraction of Exocellular Polymer and (B) the Ratio of Polysaccharide to Algal Mass.

exocellular polymer observed throughout the algal growth curve. These data show the total concentration of each component to increase during endogenous growth and the ratio of each component to algal mass to also increase during this growth phase (see Figures 7B, 8B, 9B, and 10B). Consequently, it appeared that all major components of the exocellular polymer were involved in the increase in polymer production associated with algal bioflocculation; however, it should be noted that protein and RNA appeared most prevalent in the compositional makeup of the exocellular polymer from this culture.

To substantiate bioflocculation phenomena observed in culture 2, a third culture consisting of Chlorella as the predominant algal type was investigated. Data from culture 3 (see Figures 11 through 16) closely paralleled that developed for culture 2. Inorganic contamination of extracted exocellular polymer was again observed.

The conclusions of the algal bioflocculation studies may be summarized as follows:

Algal agglutination appeared to be governed by the physiological state of the microorganisms. Algal flocculation was not observed to occur until the microbes had entered into a state of restricted growth (endogenous growth phase).

A direct correlation was found to exist between exocellular polymer production and microbial aggregation. Since exocellular polymer to algal mass ratios dramatically increased during algal flocculation, surface coverage phenomenon may be interpreted as the mechanism by which biological flocculation occurs.

Cell surface charge investigations indicated that surface potential reduction is not a necessary precursor to algal bioflocculation.

The major compositional makeup of exocellular algal constituents associated with bioflocculation may be classified into four general categories of organic polymers: polysaccharides, proteins, RNA, and DNA, thereby implying negative polymer charge characteristics.

The mechanism of algal bioflocculation is interpreted as resulting from the interaction of high molecular weight exocellular polymers, which have sufficiently accumulated at the microbial surface during endogenous growth. These

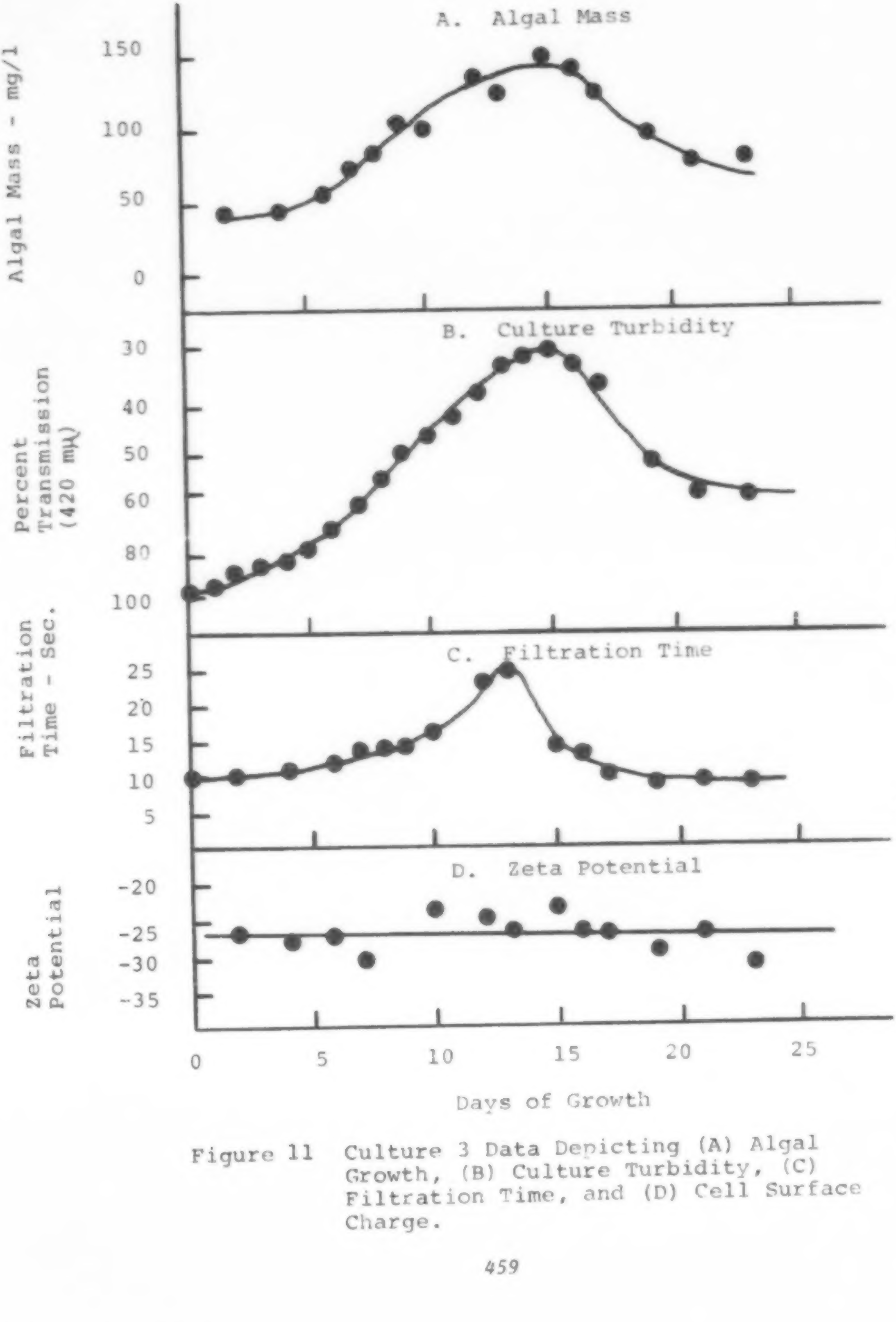


Figure 11 Culture 3 Data Depicting (A) Algal Growth, (B) Culture Turbidity, (C) Filtration Time, and (D) Cell Surface Charge.

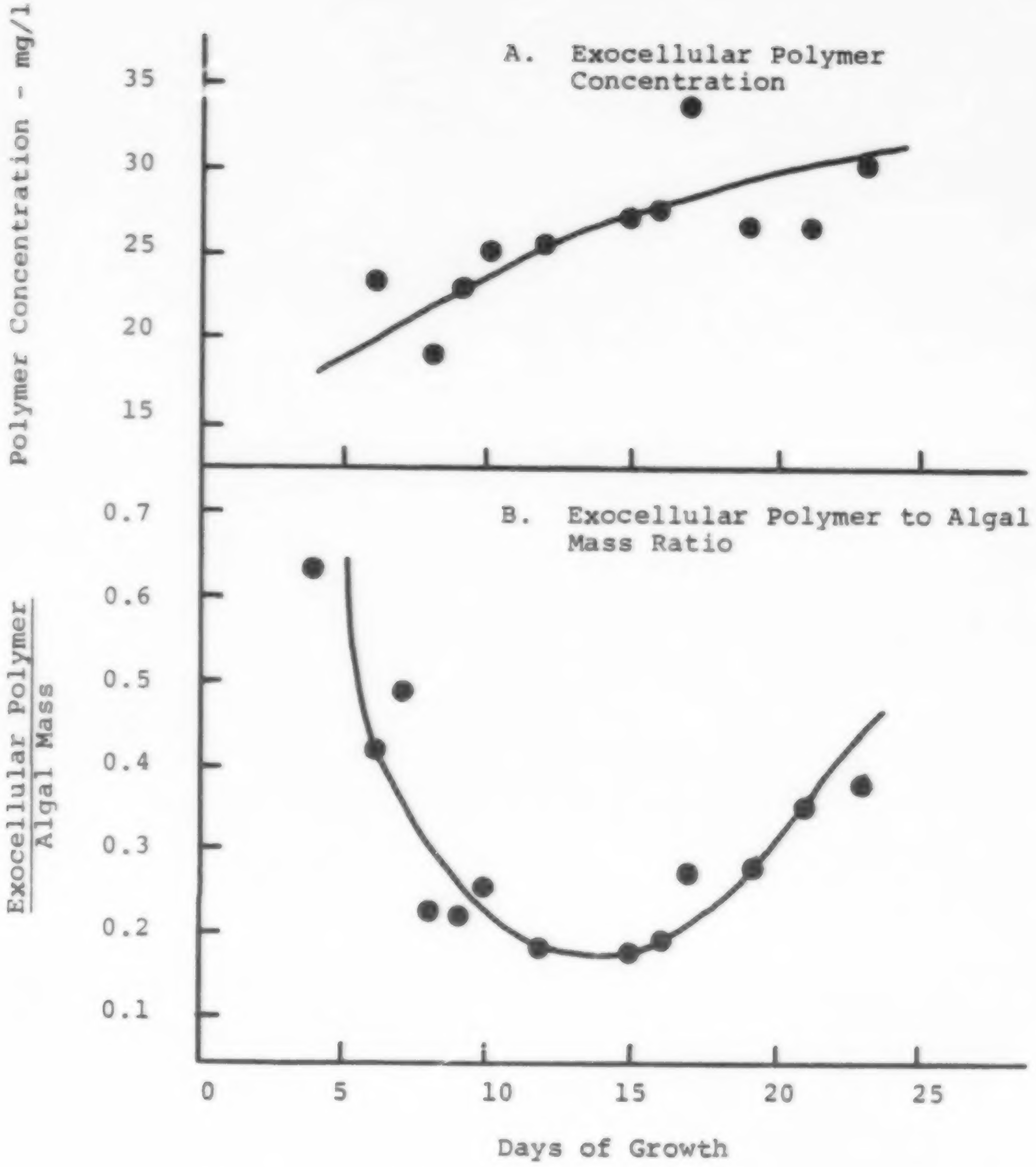


Figure 12 Culture 3 Data Depicting (A) Exocellular Polymer Production and (B) the Ratio of Exocellular Polymer to Algal Mass (from Fractional Composition Analysis).

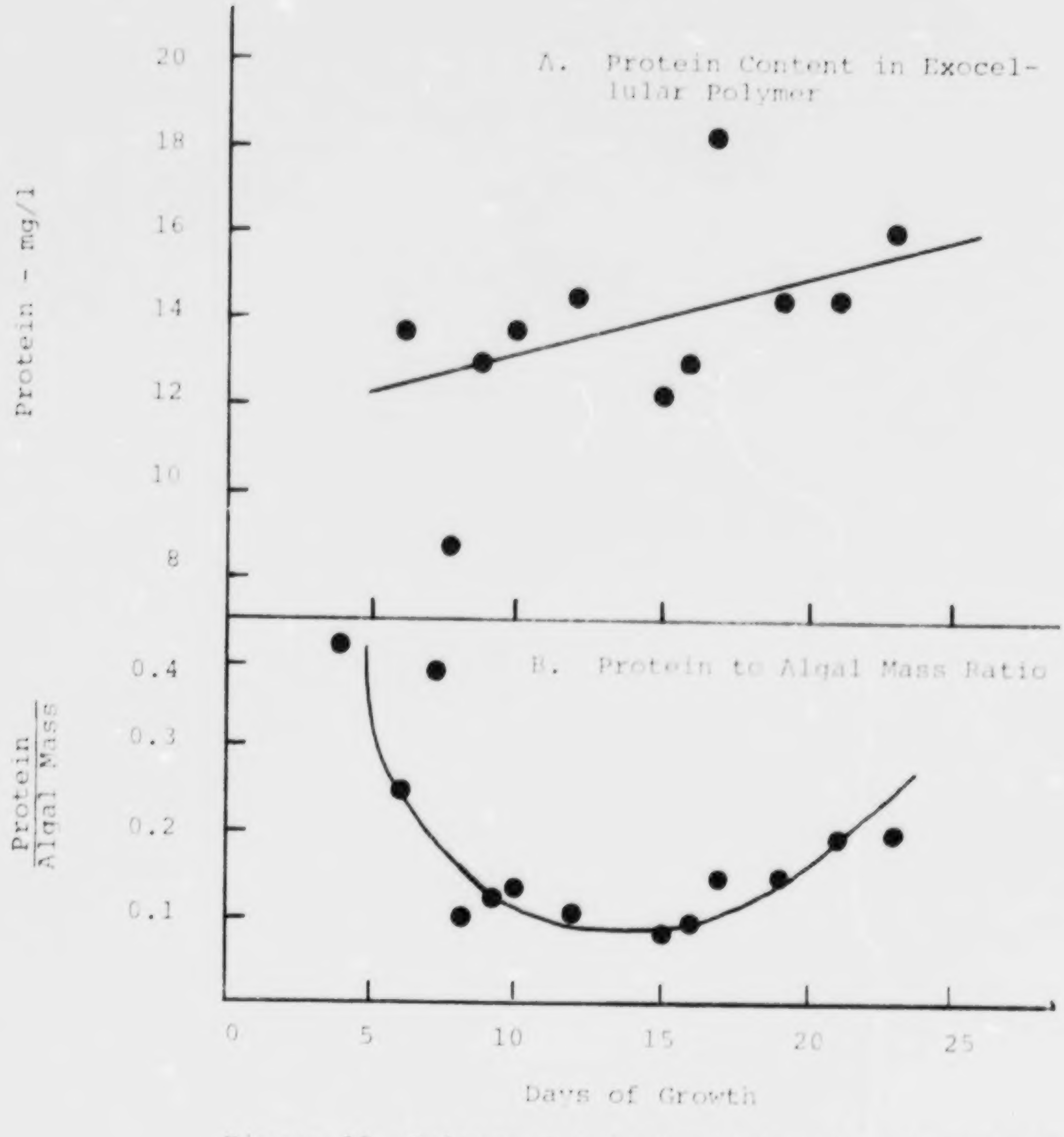


Figure 13 Culture 3 Data Depicting (A) the Protein Fraction of Exocellular Polymer and (B) the Ratio of Protein to Algal Mass.

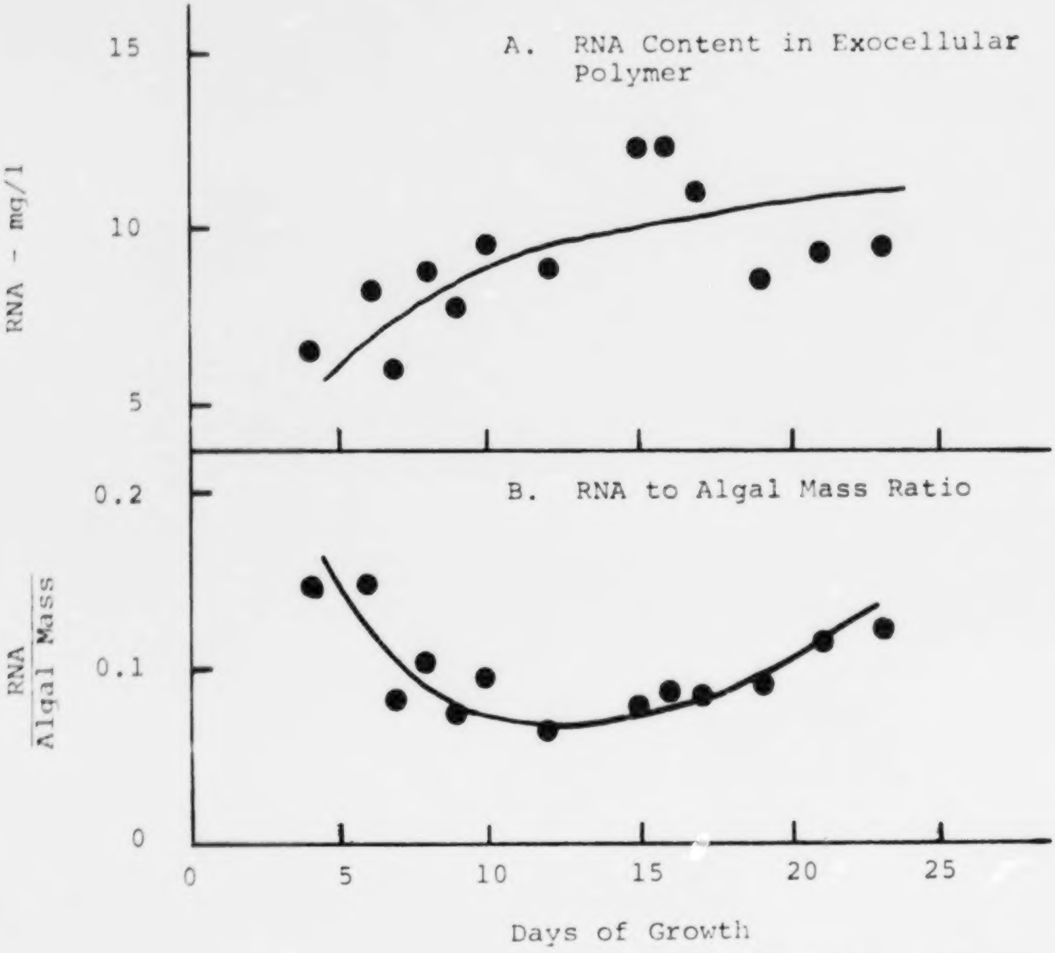


Figure 14 Culture 3 Data Depicting (A) the RNA Fraction of Exocellular Polymer and (B) the Ratio of RNA to Algal Mass.

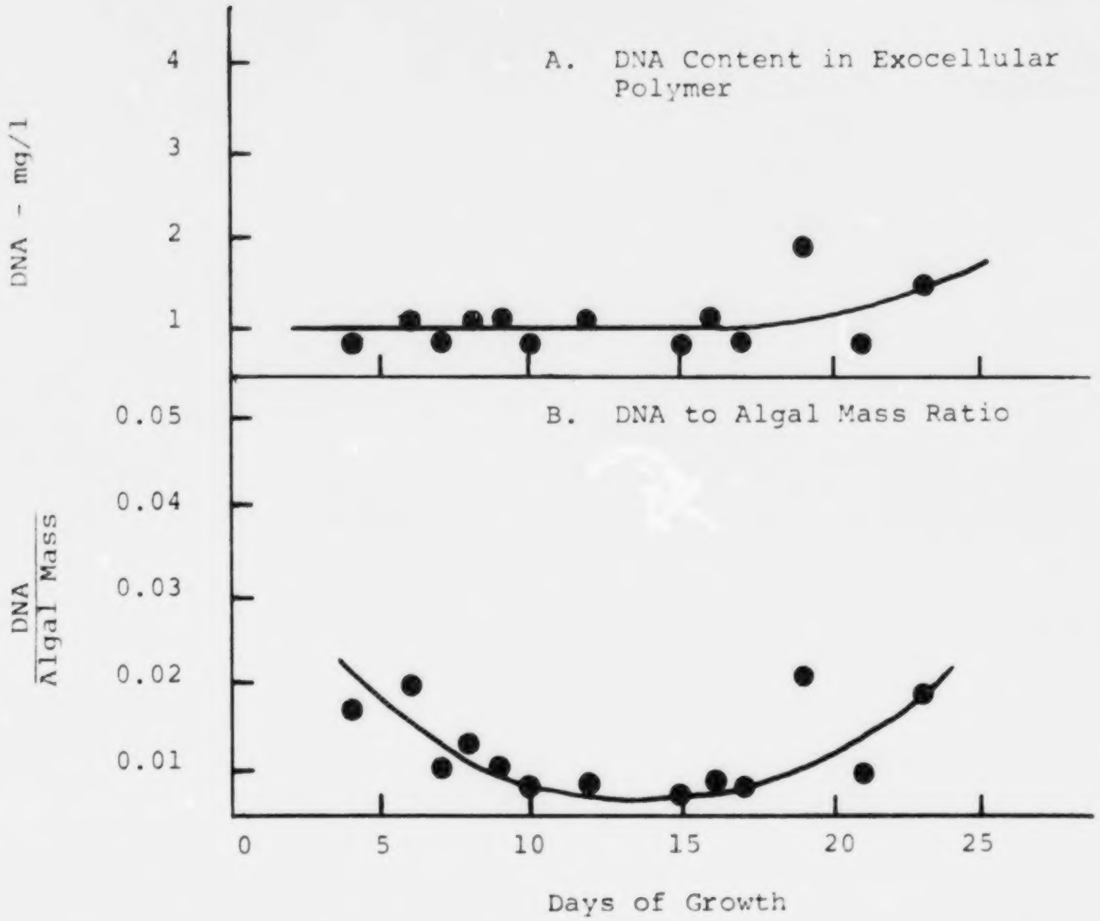
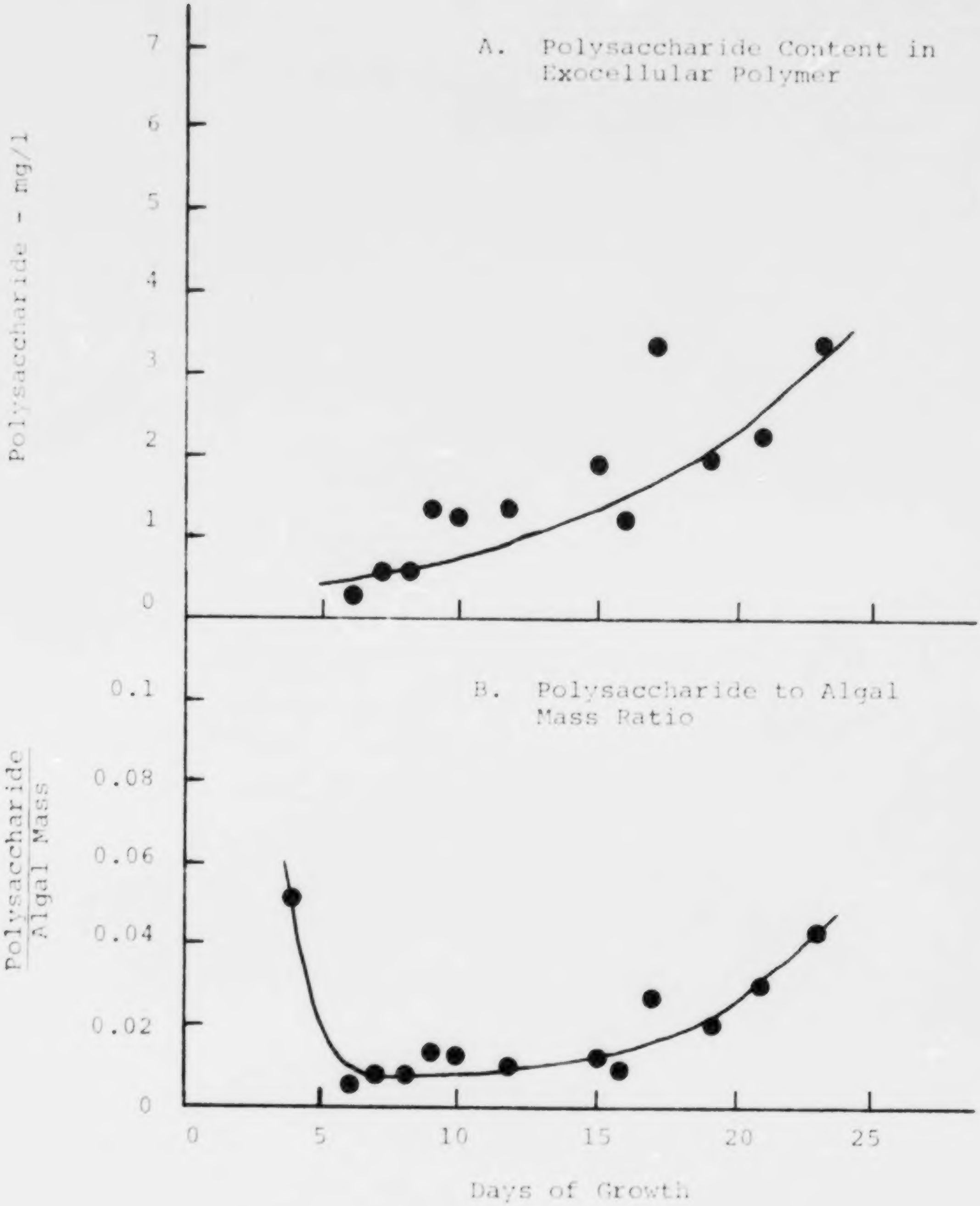


Figure 15 Culture 3 Data Depicting (A) the DNA Fraction of Exocellular Polymer and (B) the Ratio of DNA to Algal Mass.



A. Polysaccharide Content in Exocellular Polymer

B. Polysaccharide to Algal Mass Ratio

Figure 16 Culture 3 Data Depicting (A) the Polysaccharide Fraction of Exocellular Polymer and (B) the Ratio of Polysaccharide to Algal Mass.

polymers electrostatically or physically bond, and subsequently bridge, the cells of the dispersion into a three-dimensional matrix of sufficient magnitude to subside under quiescent conditions. Surface potential reduction is not necessarily a prerequisite for agglutination in this model. Algal flocculation can most conveniently be viewed in terms of surface coverage relationships.

Chemical Flocculation Studies

Batch algal reactors were utilized in this study to supply algal suspensions for chemical flocculation testing that would be similar to algal samples found in typical wastewater treatment systems. The function of the batch reactor was to simulate the growth of an incremental volume of mixed algae as it passed through an oxidation pond. The particular algal growth phase was not a major consideration in this study but rather the algal concentration at any specific time. Most chemical flocculation experiments utilized algal suspensions directly from the batch reactors with no pre-treatment to simulate as closely as possible flocculation phenomena that might be observed at an operational oxidation pond.

The composition of a "typical" algal culture utilized during the chemical flocculation investigation is given in Table 1.

Table 1. Typical Composition of
Mixed Algal Cultures Utilized
During Chemical Flocculation Studies

Broad Group	Genera	Number of Species	Percent Biomass
Green Unicellular Algae or Small Group (2-4 cells)	Chlorella	2	1
	Euglena	1	
	Scenedesmus	2	
Blue-green Filamentous	Oscillatoria	3	40
Green Filamentous	Ulothrix	2	29
Diatoms	Fragilaria	1	29
	Synedra	1	
Green Colonial	Volvox	2	1

Effect of Hydrogen Ion Concentration on Algal Autoflocculation - Before attempting to determine the feasibility of flocculating algal suspensions with synthetic organic polyelectrolytes, it was necessary to determine the effect of hydrogen ion concentration on algal autoflocculation. This data would then serve as a reference while conducting optimum pH and dosage studies for each polymer. Consequently, an algal suspension at a concentration of 166 mg/l was flocculated at varying pH levels (from 1.0 to 11.0) with no polymer addition. The resulting data, as shown in Figure 17, depicts maximum algal autoflocculation phenomena occurring at a pH of 3.0. This study indicates that natural flocculation is maximized when the surface charge of the algal biocolloid is minimized, i.e., near the isoelectric point or point of zero charge (8). At the algae's isoelectric point (pH 3.0) the electrostatic repulsive forces will be reduced to a minimum, thereby allowing the biocolloids to assume interparticle distances sufficient for van der Waal's forces to promote optimum flocculation.

Algal Flocculation with Synthetic Organic Polyelectrolytes - The polymer flocculation of algal study was intended to investigate the effectiveness of various cationic, anionic, and nonionic synthetic organic polyelectrolytes on the aggregation of various algal suspensions. The extended segment theory of polymer flocculation would predict that to achieve optimal flocculation for a given algal suspension, an optimal concentration of polyelectrolyte must be added at an optimal pH level. Addition of polyelectrolyte at pH levels other than optimum or at concentrations greater or less than the optimum will not achieve the maximum degree of flocculation. The pH will affect the surface charge density of the biocolloid and various polymer physical characteristics including linear extension, degree of ionization, and charge density. Polymer dose will control biocolloid surface coverage.

Consequently, the initial study focused on the effect of pH on the cationic polymer flocculation of algae. Standard jar tests were performed in the study at a constant cationic polymer (C-32) dosage of 2 mg/l, a constant algal concentration of 161 mg/l, and pH values varying from 1.0 to 11.0. Results indicated that optimum flocculation appeared at pH 3.0 (see Figure 18). This appears reasonable since the algal biocolloid exhibits a net charge of approximately zero at this pH level and, therefore, interparticle separation distances are at a minimum. Additionally, the cationic polymer possesses a high positive charge at this pH level and is, therefore, well extended. Similar phenomenon

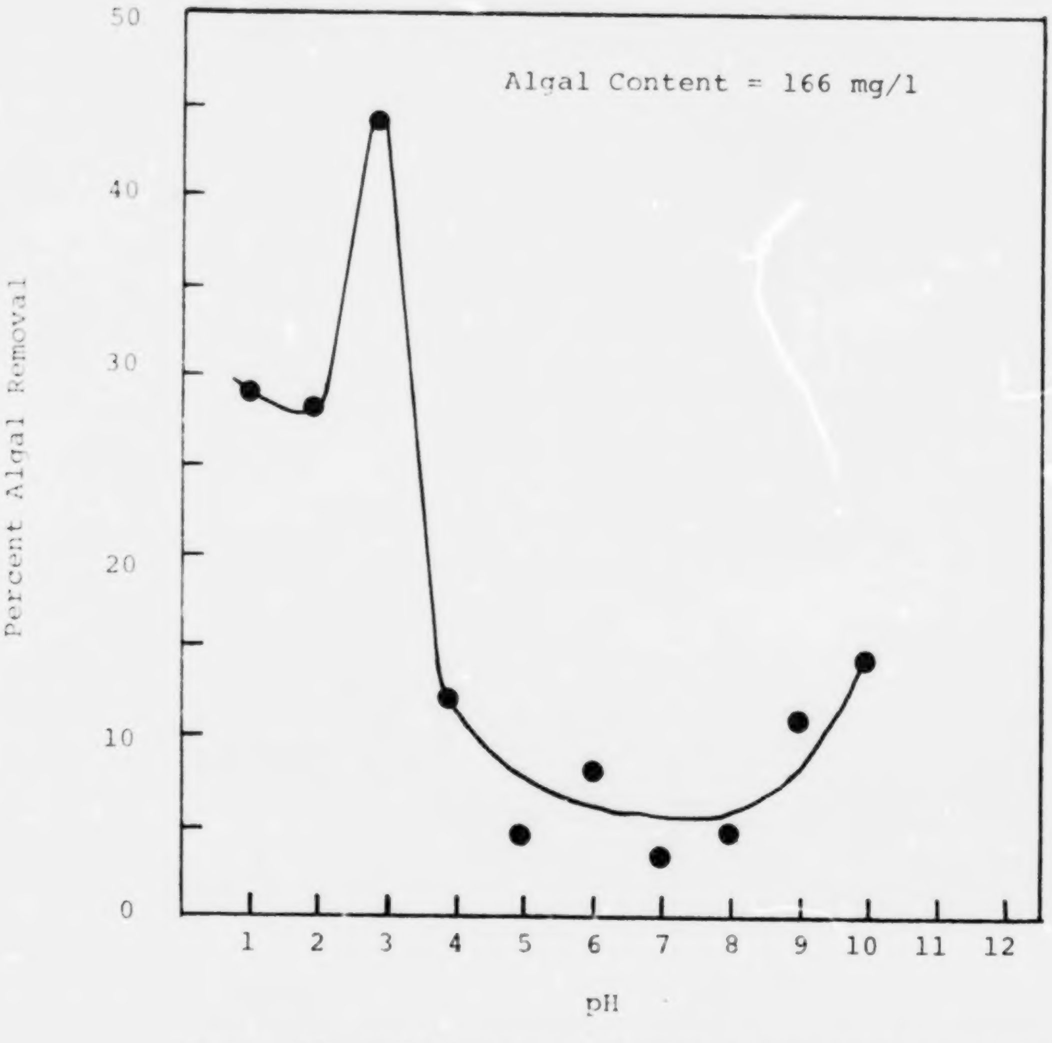


Figure 17 Relationship Between Algal Bioflocculation and Varying pH Values.

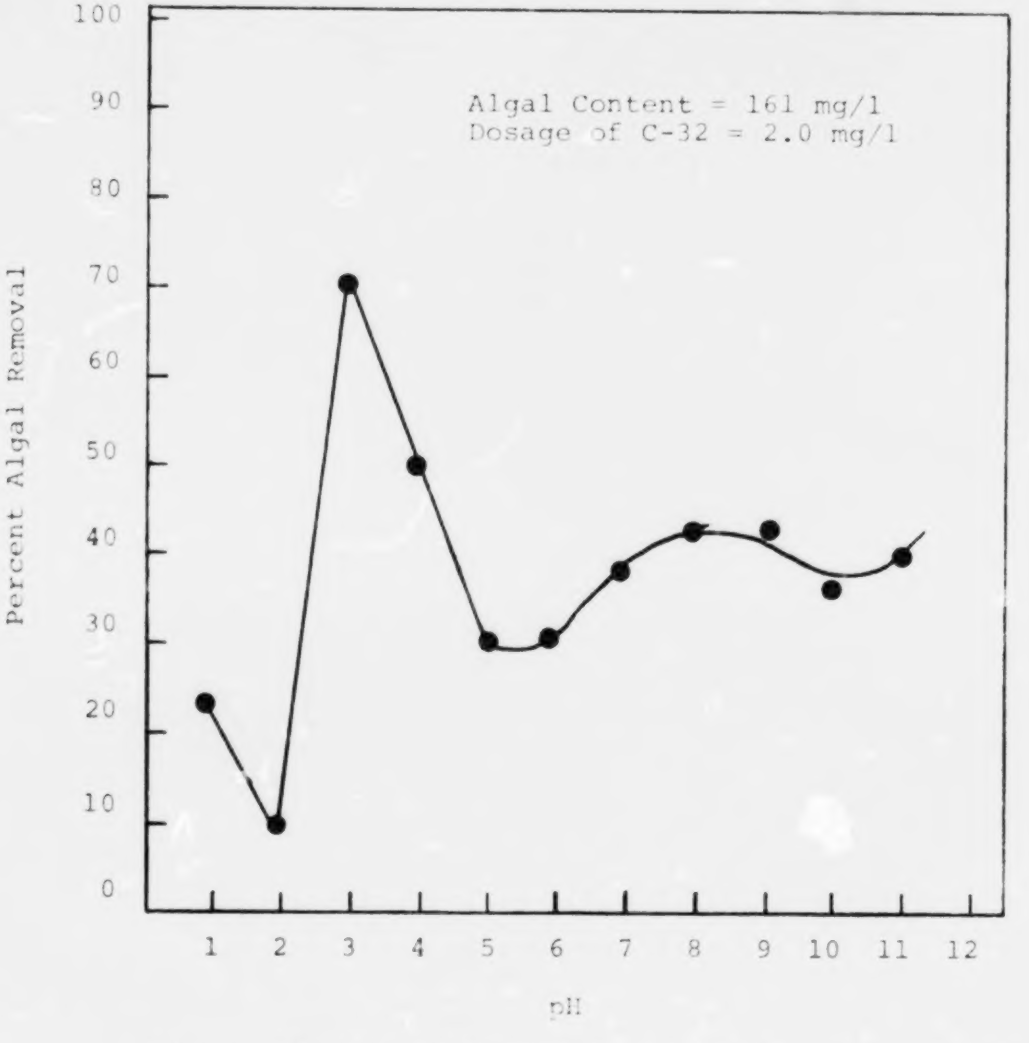


Figure 18 Algal Flocculation with Cationic Polymer C-32 at Varying pH Values.

were observed with another cationic polymer, C-31.

To determine the optimum cationic polymer dosage for algal flocculation, a study was conducted at the optimum pH (3.0), a constant algal concentration (166 mg/l), and varying dosages of C-32 (from 0 to 20 mg/l). The results, as shown in Figure 19, illustrate that optimal flocculation occurs with a C-32 dose of 1.0 to 5.0 mg/l. These data correspond to the current polymer bridging theoretical framework that states that flocculation is enhanced by polymer addition up to a certain dosage (approximately 50 percent surface coverage of the algal cell), and that addition of polyelectrolyte beyond that dosage results in restabilization of the algal biocolloid.

Anionic polymers were the next group of polymers studied. Again, standard jar testing procedures were utilized to determine the optimum pH level for anionic flocculation of algae utilizing a constant A-23 dosage of 5 mg/l, a constant algal concentration of 166 mg/l, and varying pH levels from 1.0 to 11.0. Results indicated an optimum pH of 3.0, similar to that obtained with cationic polymer systems (see Figure 20). However, whereas the mechanism of cationic polymer attachment was apparently electrostatic (due to opposite algal-polymer charges), the anionic polymer is thought to attach by chemical means, i.e., hydrogen bonding or anion interchange. It again appears reasonable that optimal flocculation phenomena occur at pH 3.0 (the algal isoelectric point) since this is the point at which the biocolloids possess a minimum separation distance. Similar pH phenomenon was observed with another anionic polyelectrolyte, A-22.

To determine the optimum anionic polymer dosage for algal flocculation, a study was conducted at the optimum pH of 3.0, a constant algal concentration of 166 mg/l, and varying A-23 dosages from 0 to 20 mg/l. The results, illustrated in Figure 21, indicate an optimum dosage of 1 to 2 mg/l A-23. Algal flocculation at the optimum A-23 dosage was only slightly improved over a system with no polymer (about 10 to 15 percent). This poor increase in flocculation efficiency appears to be due to the fact that the negative polymer is of insufficient length to bridge between the algal cells (i.e., to overcome the electrostatic repulsive separation distance and attach itself to the colloidal surface). Optimum algal flocculation most probably occurs at pH 3.0 because at this point the net negative surface charges on both the algal cell and polymer are sufficiently reduced to allow some bridging to occur. It

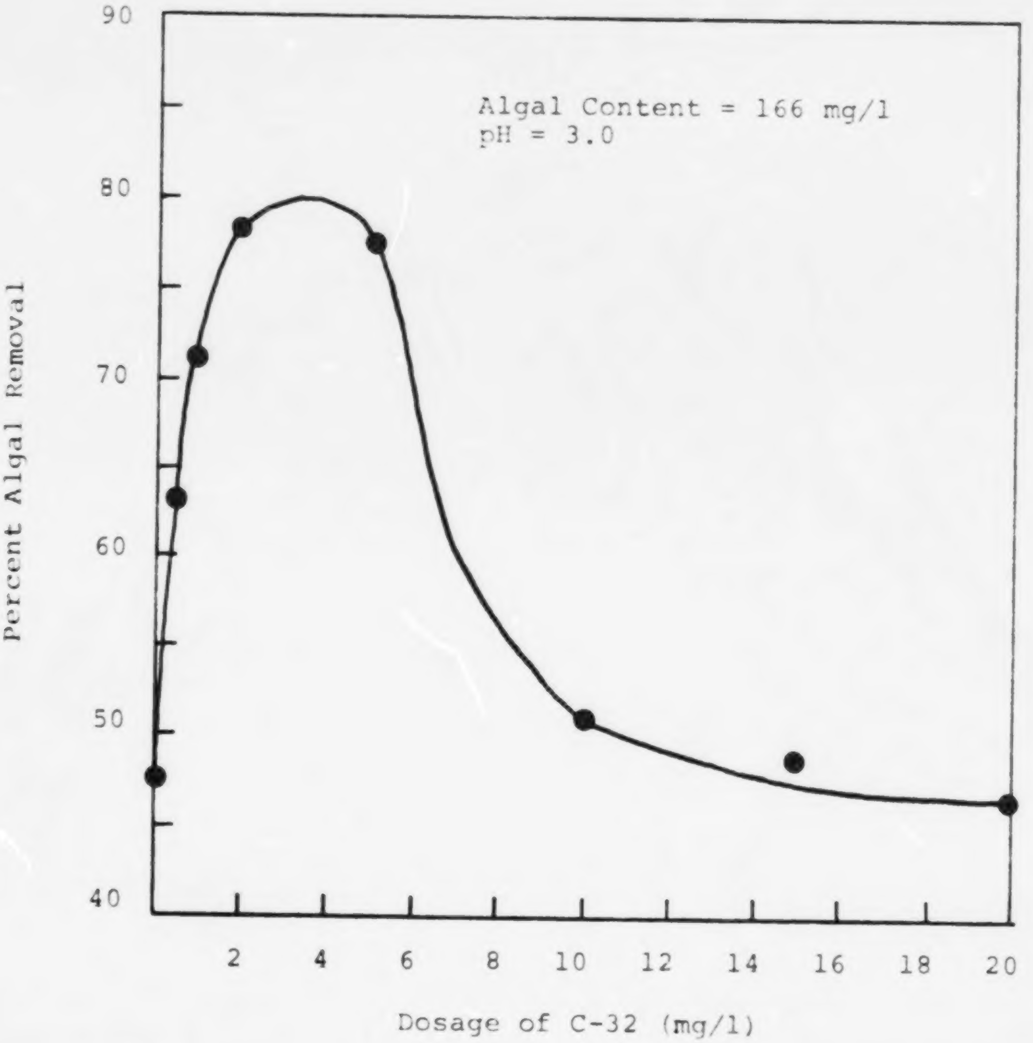


Figure 19 Algal Flocculation at Optimum pH (3.0) with Varying Dosages of Cationic Polymer C-32.

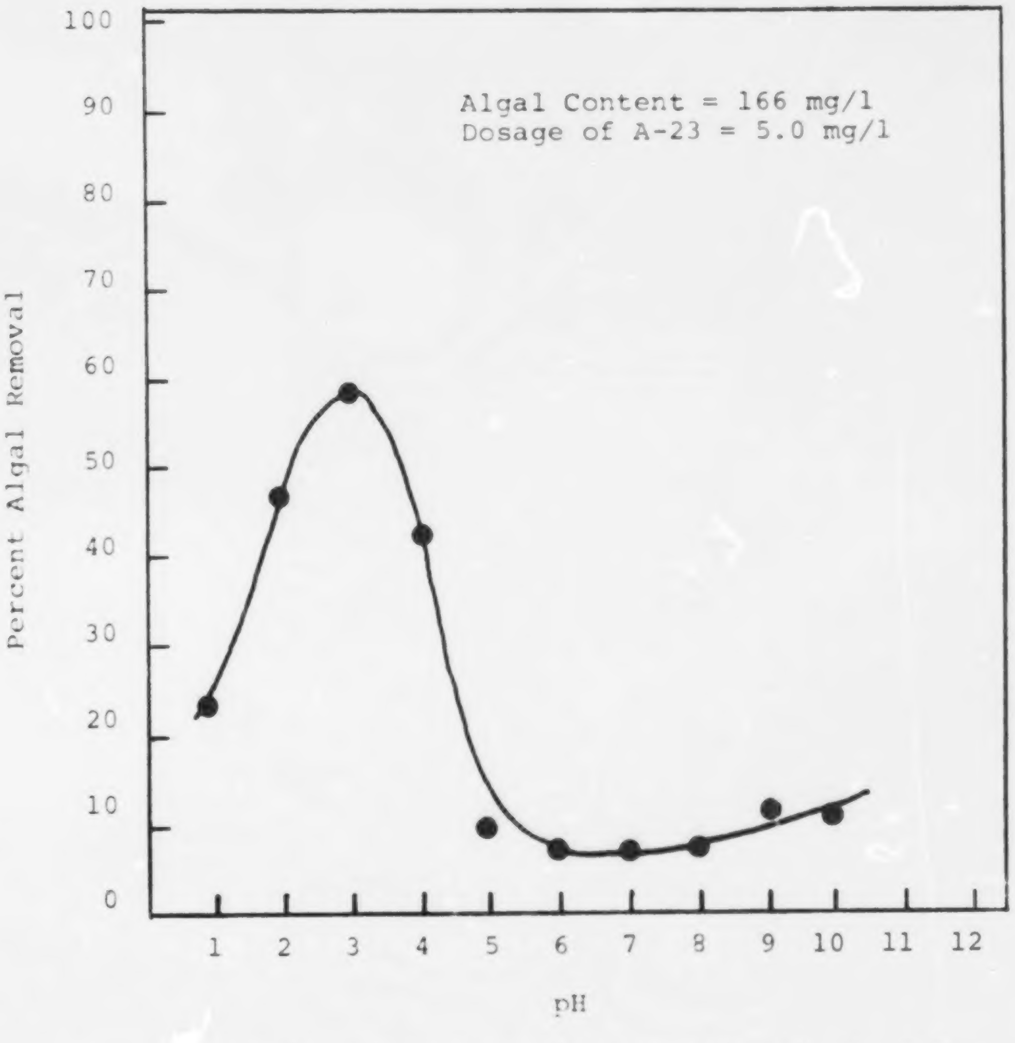


Figure 20 Algal Flocculation with Anionic Polymer A-23 at Varying pH Values.

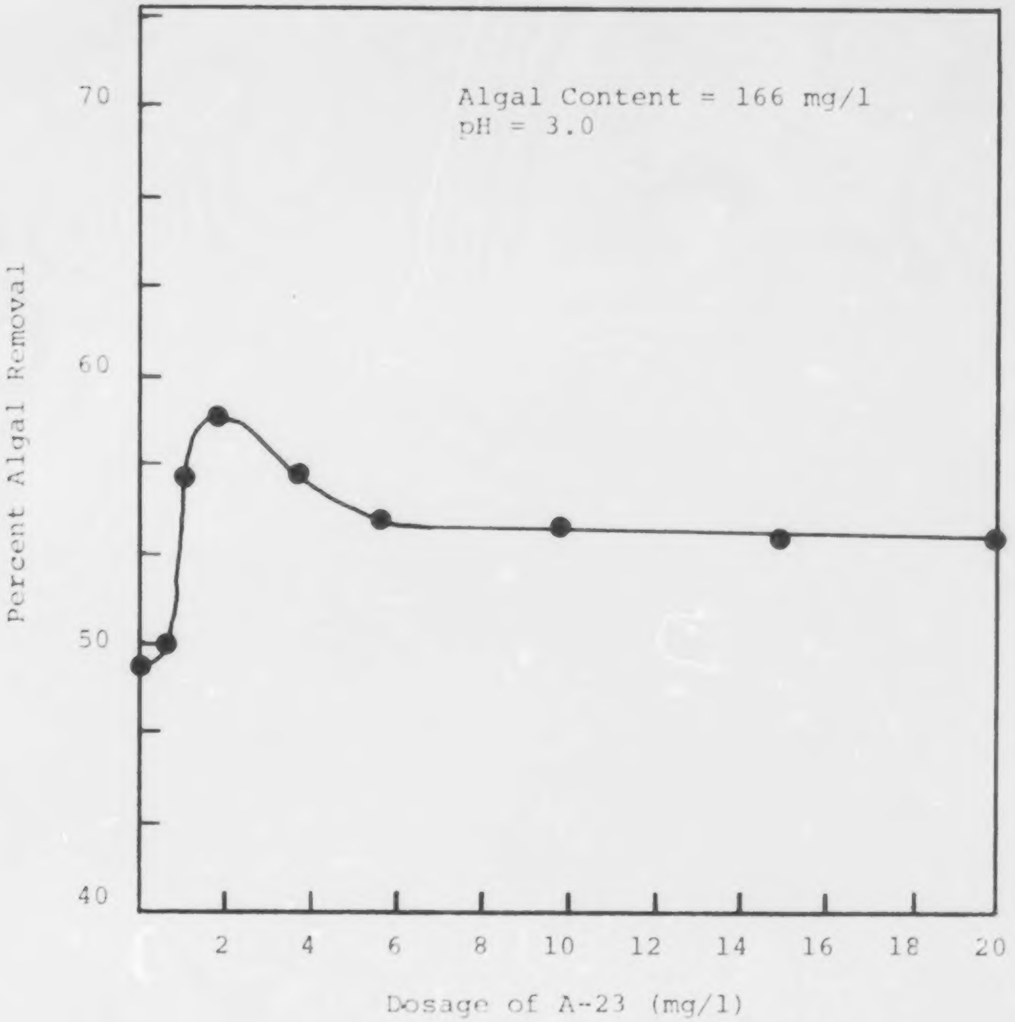


Figure 21 Algal Flocculation at Optimum pH (3.0) with Varying Dosages of Anionic Polymer A-23.

should be noted that similar flocculation phenomena were observed with another anionic polymer, A-22.

Optimum pH and polymer dosage studies were also performed with nonionic polymers, i.e., polymers having a net charge of zero. To determine the optimum pH for algal flocculation with a nonionic polymer, N-12, a standard jar test was performed at a constant algal concentration of 153 mg/l, a constant N-12 dosage of 2.0 mg/l, and varying pH values from 1.0 to 11.0. The results of this study again indicated an optimum pH of 3.0 (see Figure 22). The poor flocculation achieved with the nonionic polymers can again be attributed to insufficient polymer chain length. Optimum flocculation again occurs at the point where the electrostatic repulsion forces are minimum and, therefore, allow some polymer bridging to occur. At the elevated pH ranges the repulsive forces produce biocolloid separation distances large enough to prevent polymer adsorption onto the surface of the algal cell.

To determine the optimum dose of N-12 for algal flocculation, a study was conducted at the optimum pH (3.0), a constant algal concentration of 171 mg/l, and a polymer dosage varying from 0 to 20 mg/l. The results, shown in Figure 23, indicate an optimum dosage of 5.0 mg/l. This nonionic polymer at its optimum dosage achieved an increase in algal removal over that achieved by the anionic polymers. However, it was again postulated that the nonionic polymers were of insufficient length to bridge the electrostatic separation distance effectively. Although the biocolloid-nonionic polymer repulsion forces are assumed to be minimal due to the net nonionic polymer charge of zero, the surface charges of both the polymer and algal cell still appear to exert a significant effect upon algal flocculation with nonionic polymers. It should be noted that similar flocculation data was developed with two other nonionic polymers, N-11 and N-17.

In summary, Figure 24 illustrates the general effect of pH upon algal flocculation with various polymers. With each polymer tested the flocculation phenomenon followed the same general pattern. The major factor controlling these results appears to be the isoelectric point (i.e., point of zero charge) of the algae which occurs at a low pH level and dominates polymer flocculation mechanisms. The effect of optimum polymer dosage followed a pattern in that dosages beyond the optimum resulted in decreased algal removal. This effect appears to arise from biocolloid restabilization due to excessive surface coverage with

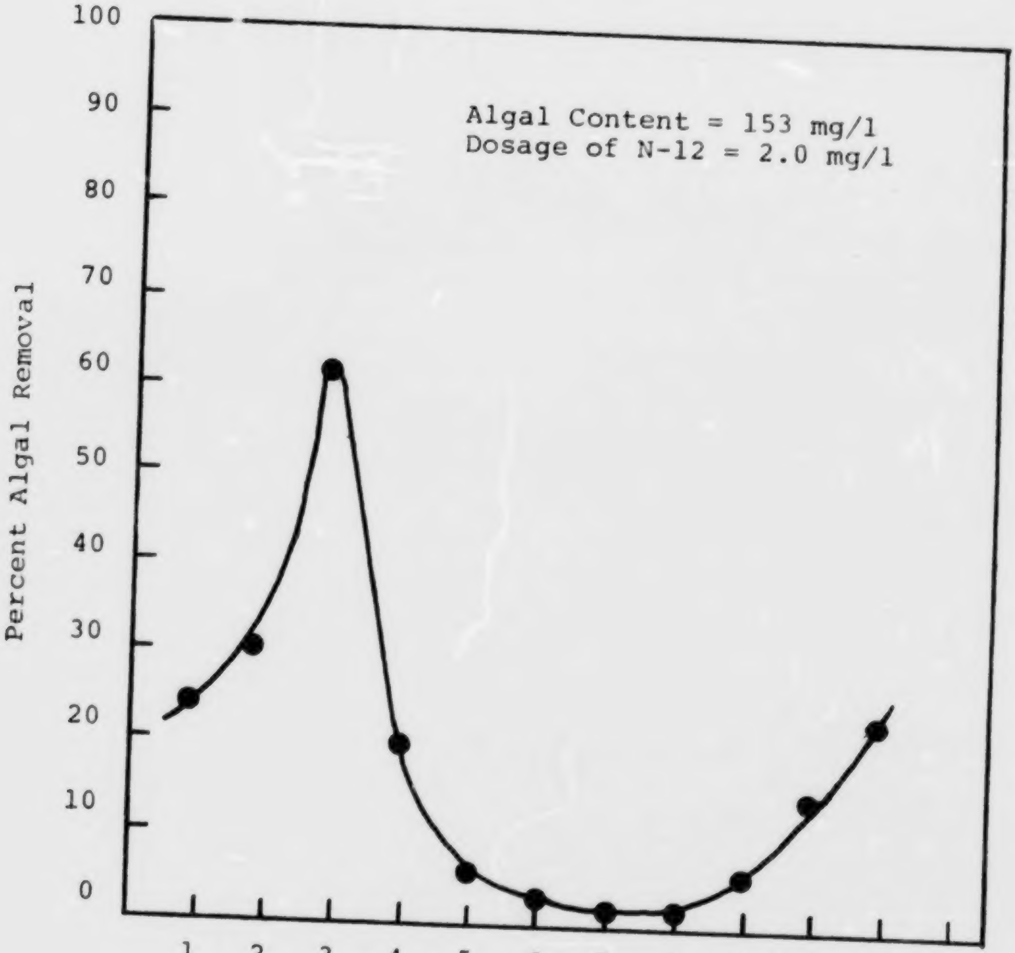


Figure 22 Algal Flocculation with Nonionic Polymer N-12 at Varying pH Values.

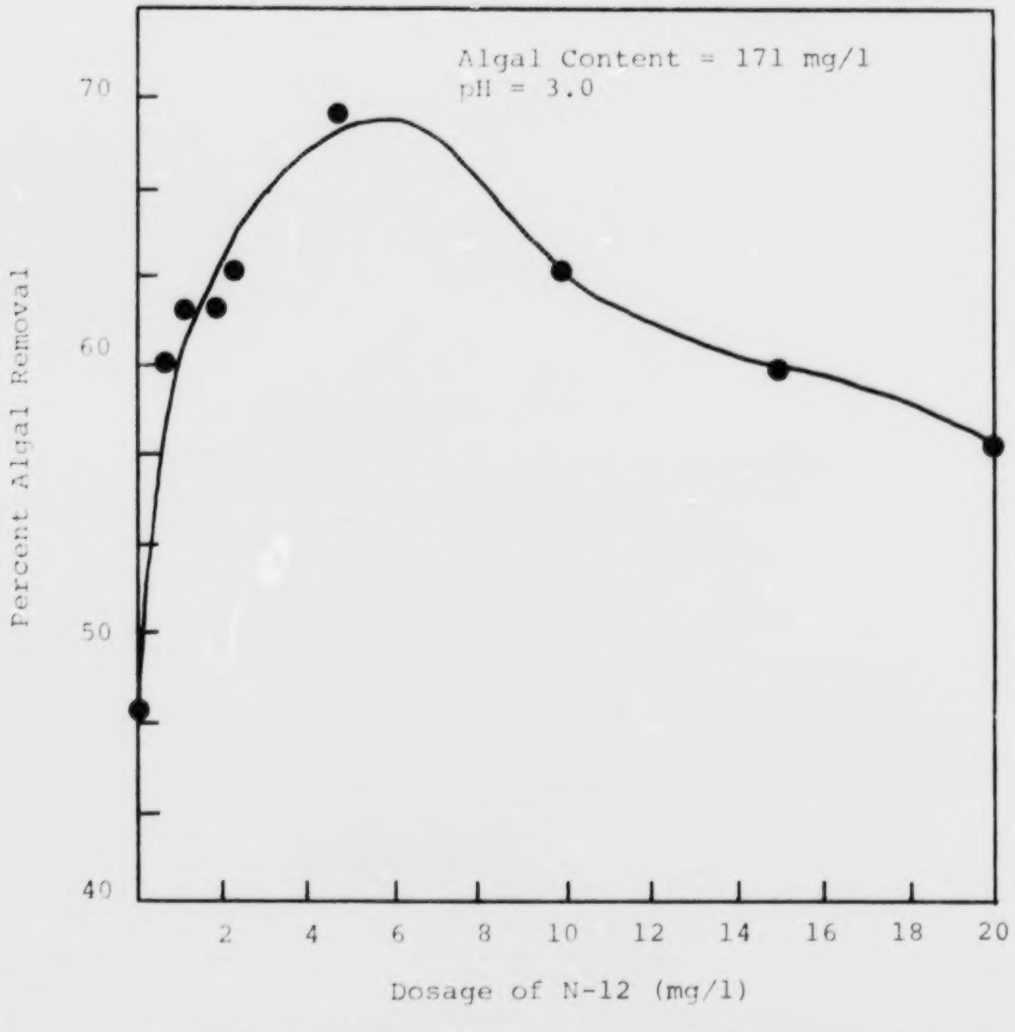


Figure 23 Algal Flocculation at Optimum pH (3.0) with Varying Dosages of Nonionic Polymer N-12.

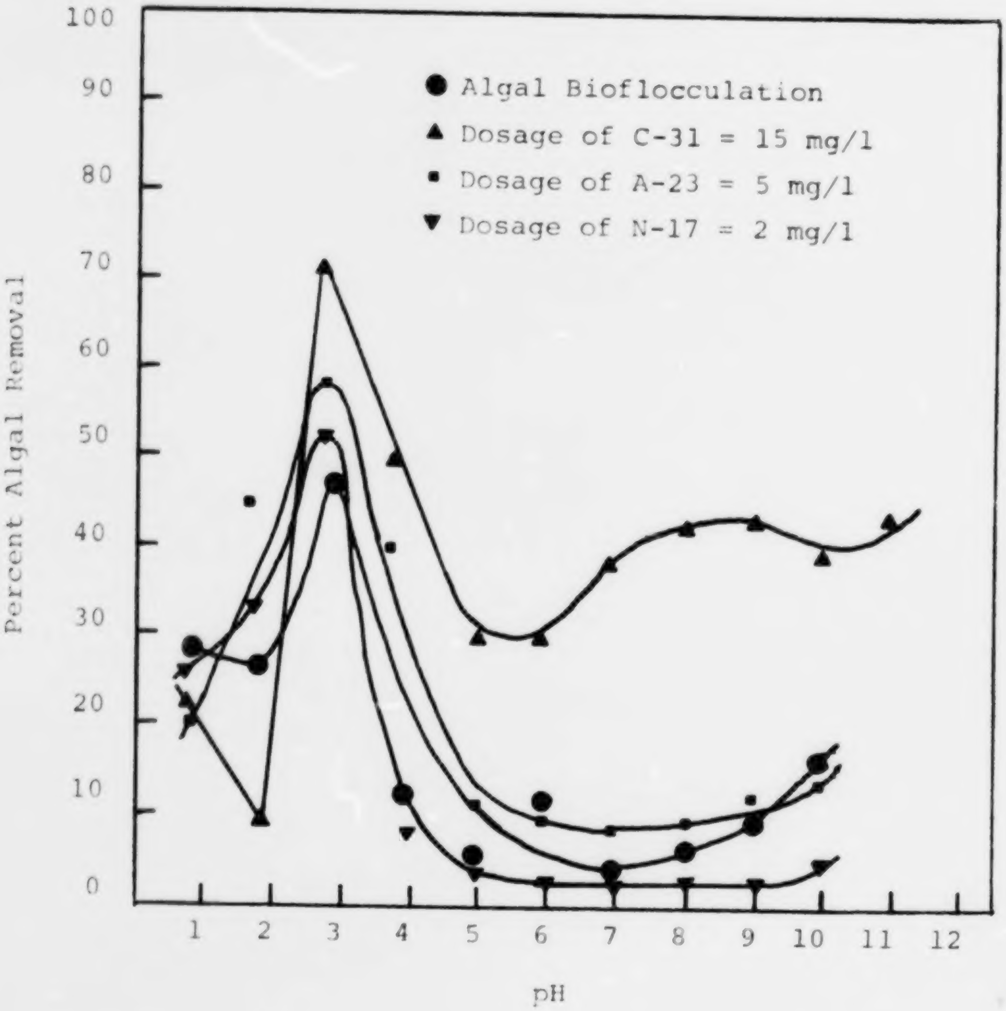


Figure 24 Effect of Varying pH Values on Algal Flocculation Synthetic Polymers.

polymer.

Algal Flocculation at Naturally Occurring pH Levels - Since the majority of naturally occurring biological systems operate in the neutral pH ranges, it was decided to conduct some polymer flocculation tests at pH 7.0. The initial study conducted at pH 7.0 was to determine the efficiency of algal flocculation with cationic polymers C-31 and C-32. Results indicated optimum C-31 and C-32 polymer dosages of 15 mg/l and 20 mg/l, respectively (see Figure 25). The significant difference between these tests and the ones previously conducted at the optimum pH of 3.0 is that the dosages required for optimal algal flocculation are considerably increased. This is probably due to increased biocolloidal electrostatic repulsive forces since the algal biocolloid becomes more negative as the hydrogen ion concentration decreases. Also, the cationic polymer will be less extended at higher pH values.

Algal flocculation studies were also performed with certain anionic and nonionic polymers at pH 7.0 to determine the effect of neutral pH levels on algal removal with these polymers. The results of these studies, as shown in Figure 26, indicate that very little polymer bridging is occurring probably due to insufficient polymer length. Also, the electrostatic repulsive forces are significantly increased on the algal surface at pH 7.0 compared to pH 3.0. These factors plus the inability of anionic and nonionic polymers to perform a charge neutralization function result in poor algal removal.

Algal Flocculation with Polymers and Trivalent Metal Ion Additives - The remainder of this investigation concentrated on algal removal with anionic polyelectrolytes, in particular Dow Purifloc A-23. Since in the previous studies flocculation was not achieved with polymer addition alone, it was decided to add a trivalent metal ion to act as a bridge between the negative algal particle and the negative polymer. It was felt that the trivalent metal ion could achieve one or both of the following functions. First, the metal ion could reduce the algal cell surface potential, thereby allowing the negative polymer to overcome the electrostatic separation distance and attach itself to the algal surface, thereby allowing bridging between the various cells. Or secondly, it was felt that the metal ion could serve as a bridge between the algal cell and anionic polymer forming a biocolloidal-metal ion-polymer-metal ion-biocolloid complex. If either or both mechanisms were achieved, good settling was expected to result.

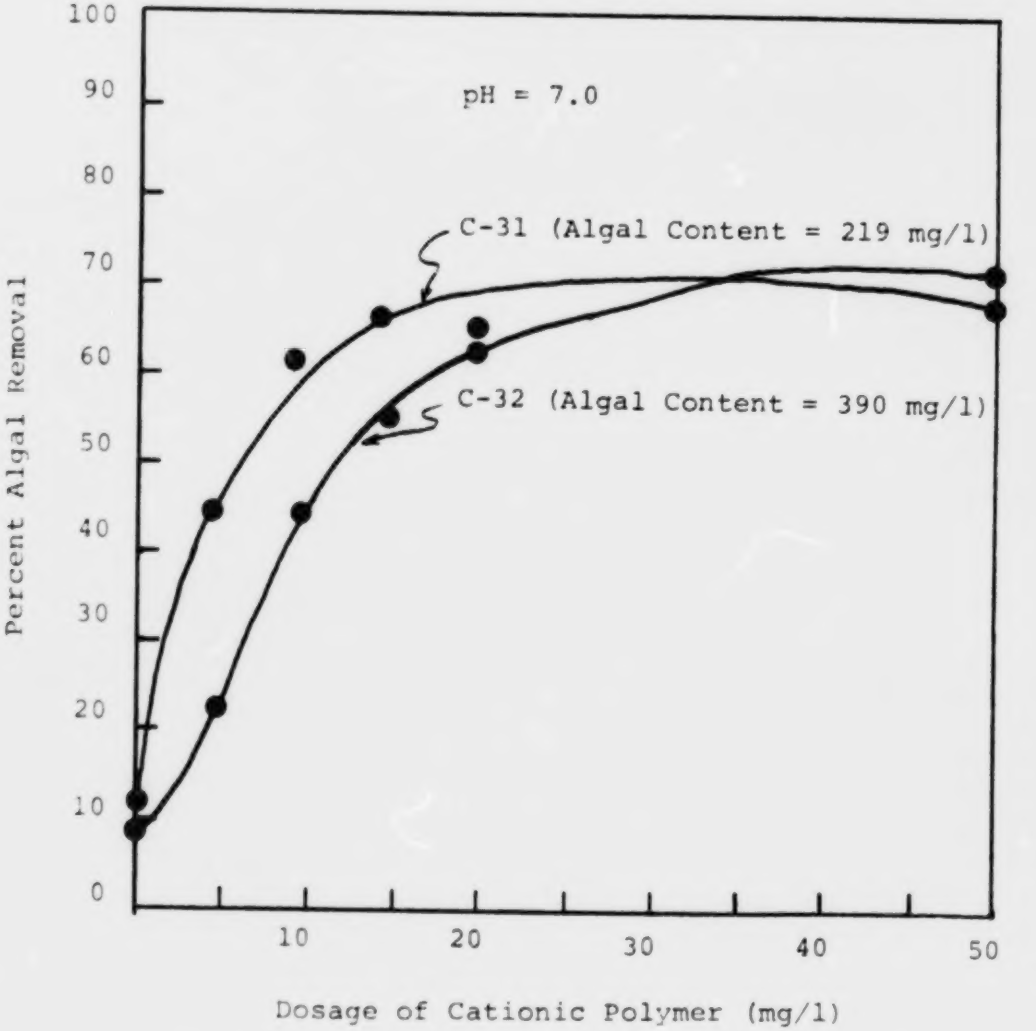


Figure 25 Algal Flocculation with Cationic Polymers C-31 and C-32 at pH 7.0.

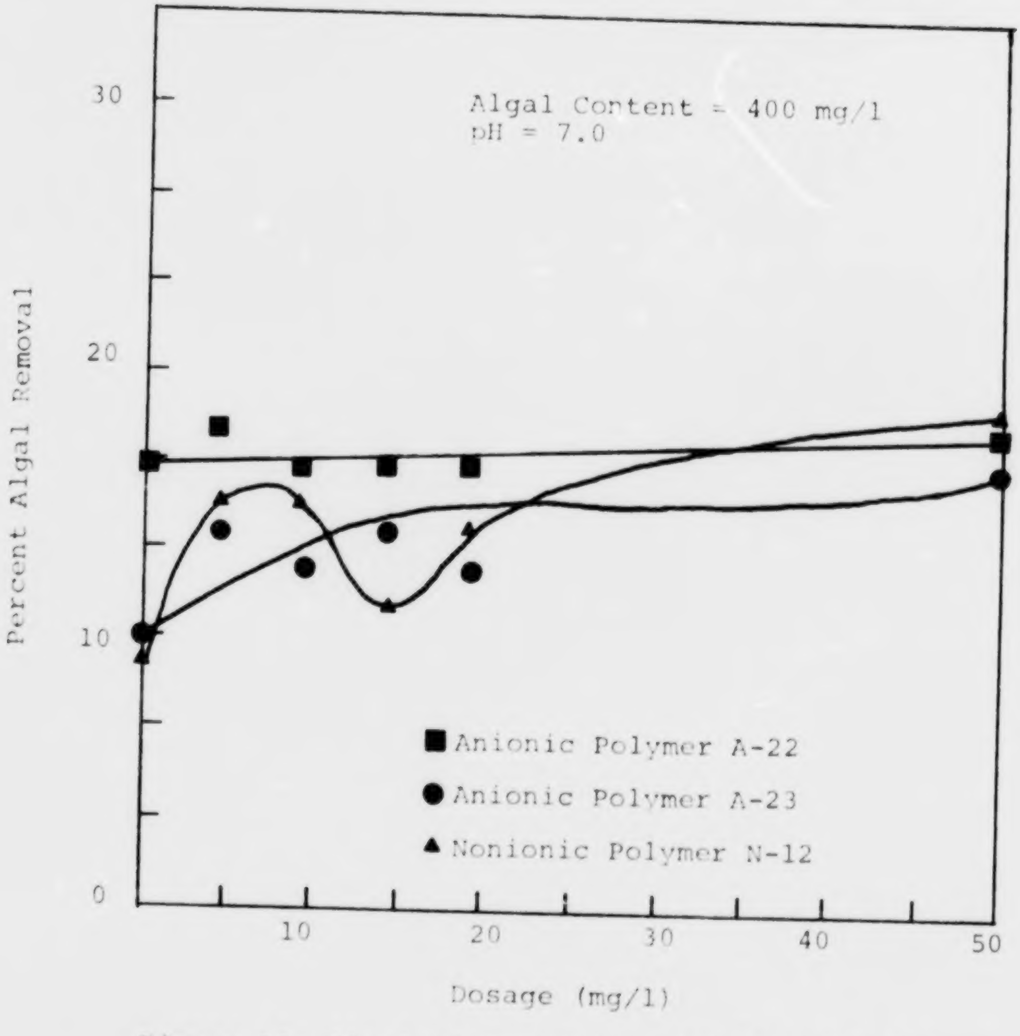


Figure 26 Algal Flocculation with Anionic and Nonionic Polymers at pH 7.0.

Alum (aluminum sulfate) was chosen as a representative metal ion because of its wide use in the field of water and wastewater treatment. The initial study utilizing alum involved the addition of varying alum concentrations (from 0 to 100 mg/l as Al^{3+}) to algal samples at a constant pH of 7.0 and a constant algal concentration of 170 mg/l. The results indicated an optimum dose in the range of 15 to 20 mg/l as depicted in Figure 27. Algal flocculation was achieved primarily by charge neutralization with the positive aluminum metal ion. If the positive metal ion is added in excess of the optimum dosages, it can cause restabilization of the colloidal suspension. This occurs by adsorption of the positive metal ion into the diffuse layer around the algal cell in such quantities that the net charge on the particle becomes positive, thus causing electrostatic repulsion. The optimum dosage of the metal ion occurs when adsorption of the positive ion has reduced the surface potential to its lowest point, thereby allowing van der Waal's forces to dominate and cause flocculation.

To determine algal removal phenomena with A-23 and alum, a study was conducted at the optimum alum dosage (20 mg/l), a constant pH (7.0), a constant algal concentration of 176 mg/l, and a varying A-23 dosage (from 0 to 20 mg/l). The results, depicted in Figure 28, illustrate an optimum A-23 dose of 1.0 mg/l. The removal of algae was considerably improved over a similar system without the alum addition (see Figure 26). This would indicate that the metal ion is capable of serving as a bridge between the negative algal colloid and the negative polymer. Also, the alum-polymer system improved flocculation over that of alum alone (see Figure 26).

The second trivalent metal ion utilized was Fe^{3+} in the form of ferric chloride. An initial study was performed to determine the optimum iron dosage. This test was conducted on algal samples at a constant concentration of 166 mg/l, a constant pH of 7.0, and an iron dosage varying from 0 to 100 mg/l as Fe^{3+}. The results, as shown in Figure 29, indicate an optimum iron dosage of 50 mg/l. Iron dosages above 50 mg/l caused restabilization of the biocolloids and thus hindered the flocculation process. This phenomenon is similar to that observed with the aluminum.

To determine the optimal A-23 dose for algal removal with an iron additive, a study was conducted as a constant pH of 7.0, a constant algal concentration of 170 mg/l, a con-

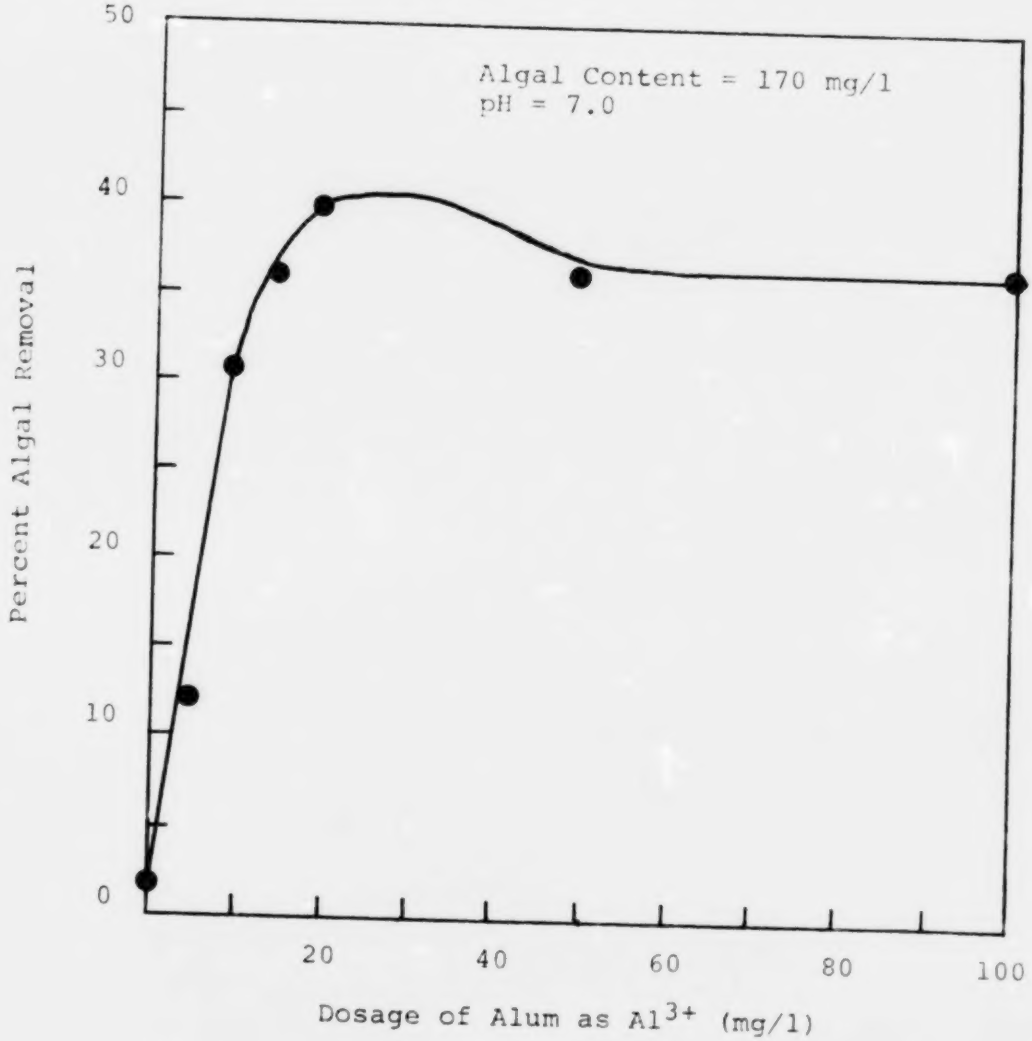


Figure 27 Algal Flocculation with Varying Dosages of Alum at pH 7.0.

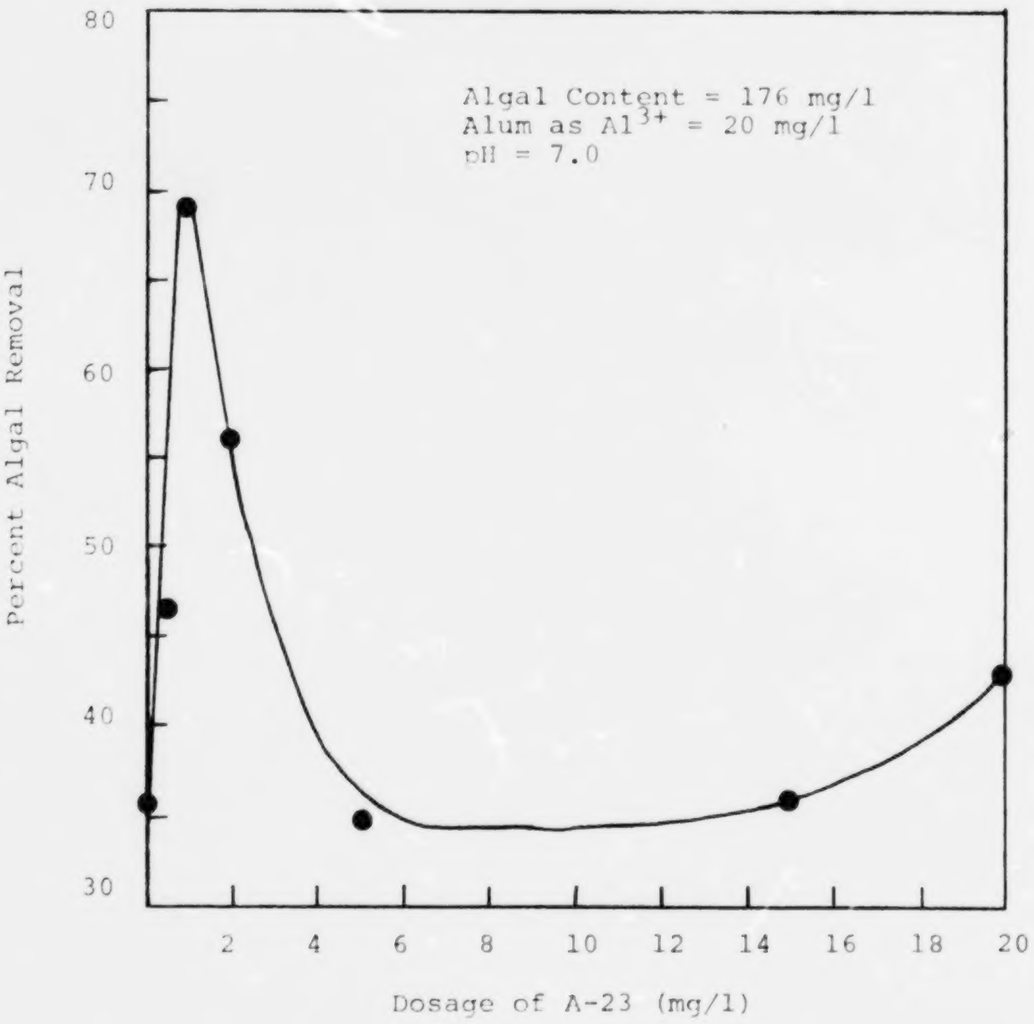


Figure 28 Algal Flocculation with Optimum Alum Dosage (20 mg/l) and Varying Dosages of Anionic Polymer A-23 at pH 7.0.

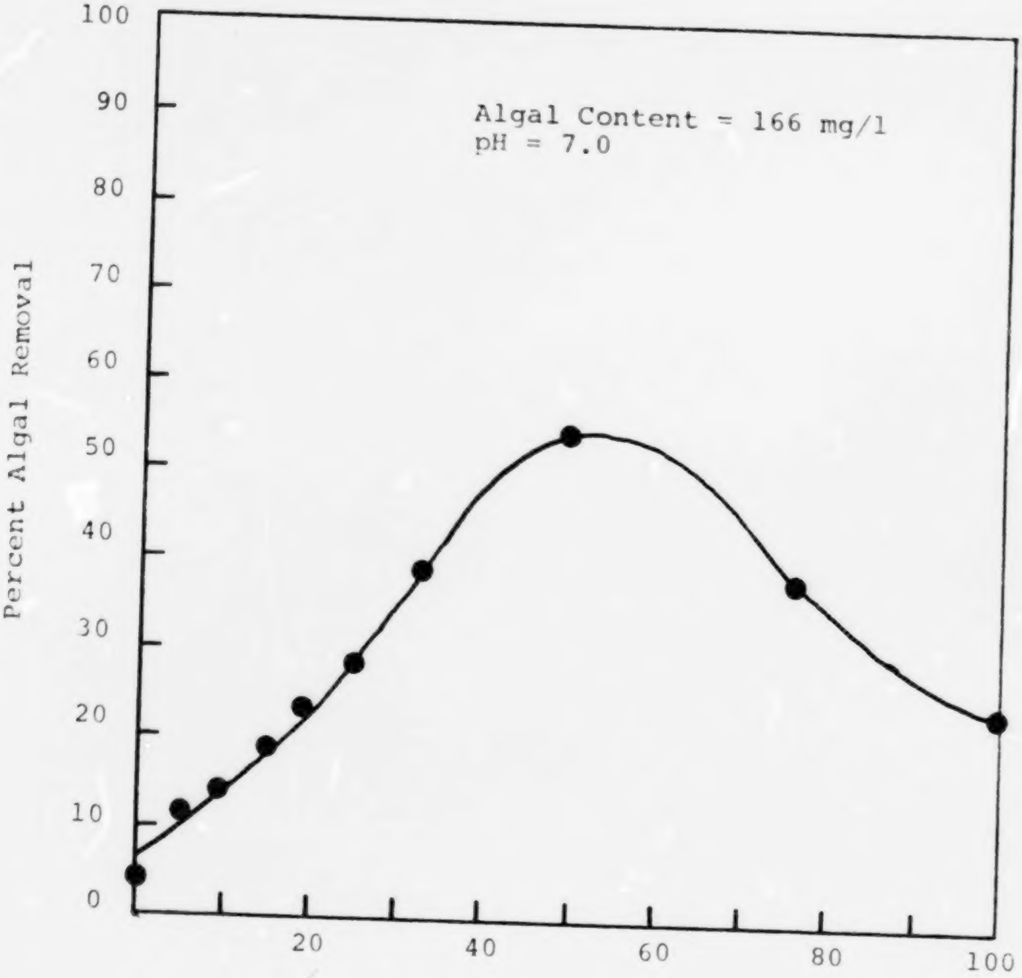


Figure 29 Algal Flocculation with Varying Dosages of Iron at pH 7.0.

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stant Fe^{3+} dosage of 50 mg/l, and varying dosages of A-23 ranging from 0 to 20 mg/l. The results, depicted in Figure 30, show that over 90 percent algal removal is achieved with an A-23 dose of 1.0 mg/l. Although the algae was effectively removed with the addition of the Fe^{3+} and A-23, the mechanism of removal did not appear to be solely that of polymer bridging. Due to an excess of phosphate ion in the culture medium, it appeared that the Fe^{3+} was precipitating the phosphate causing an enmeshment of algae and polymer in the iron-phosphate precipitate. Restabilization of the biocolloidal system did not seem apparent from the results but would probably occur at excessively high polymer dosages. Even though these results depict effective algal removal, such removals would not be achieveable in actual systems since naturally occurring algal systems usually contain about no more than 4 mg/l to 5 mg/l of phosphorus, whereas the culture media contained several hundred mg/l of phosphorus. Therefore, the next series of studies were performed to determine whether or not these high removals could be achieved in a harvested algal culture.

The first investigation conducted on a harvested culture (i.e., a culture with a phosphate content less than 1.0 mg/l) was to determine the optimum iron dosage. A study was conducted at a constant pH (7.0), a constant algal concentration (166 mg/l), and varying dosages of Fe^{3+} (ranging from 0 to 500 mg/l). The results, shown in Figure 31, indicate an optimum iron dosage of 200 mg/l. The algal removal curve again shows a peak with decreased removals occurring when iron is in excess of the optimum. However, the significant factor is that the optimum iron dosage is considerably higher (4 times as great) in the harvested suspension as opposed to the "dirty" suspension. This would seem to indicate that algal enmeshment in the iron-phosphorus precipitate did occur in the previous study. Also, the percent algal removal achieved by the iron at the optimum dose was reduced by about 50 percent as compared to the removals achieved in the "dirty" suspension (see Figure 29).

The next investigation was intended to determine the percent algal removal with A-23 and iron in the harvested culture. A study was performed at a constant algal concentration (166 mg/l), a constant pH (7.0), the optimum iron dosage (200 mg/l as Fe^{3+}), and varying dosages of A-23 varying from 0 to 20 mg/l. The results depict an optimum A-23 dosage occurring at 0.5 mg/l with subsequent reduction in percent removals due to excessive A-23 doses

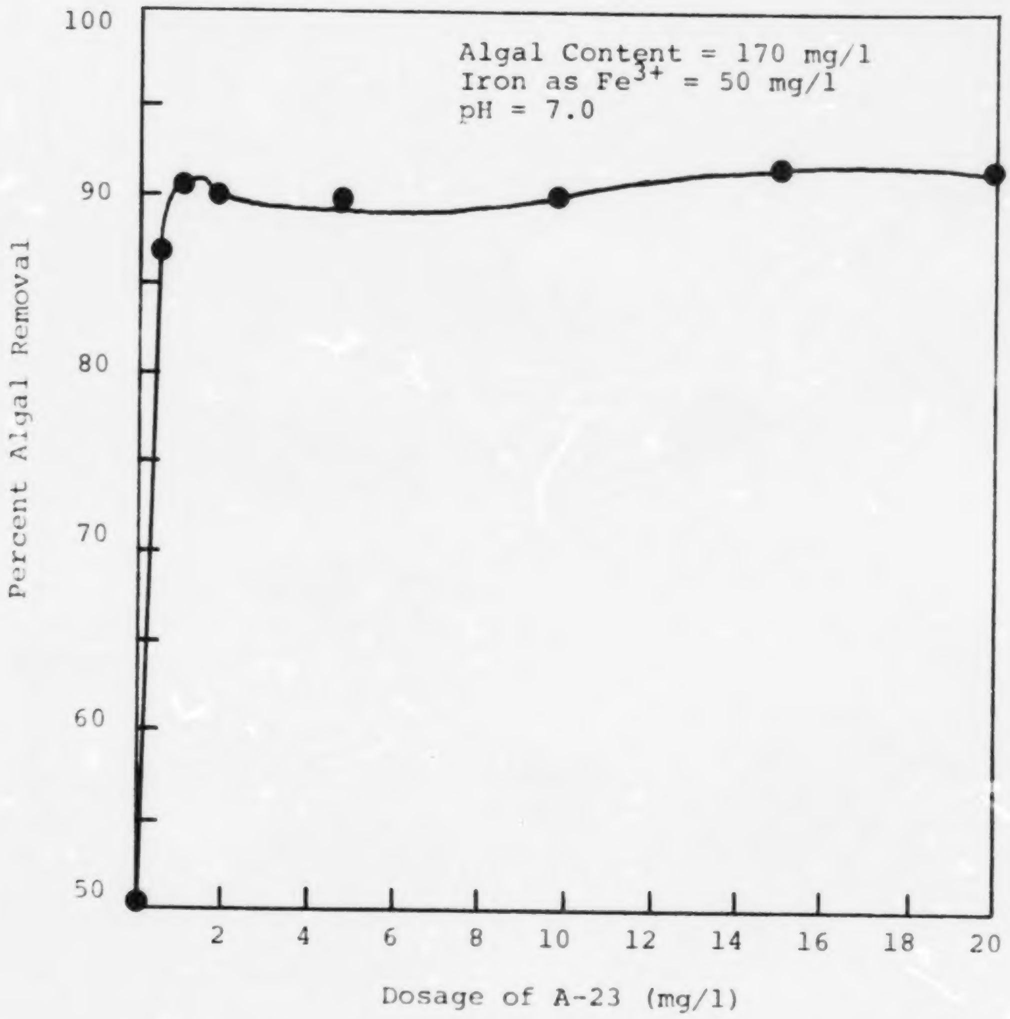

Figure 30 Algal Flocculation with Optimum Iron Dosage (50 mg/l) and Varying Dosages of Anionic Polymer A-23 at pH 7.0.

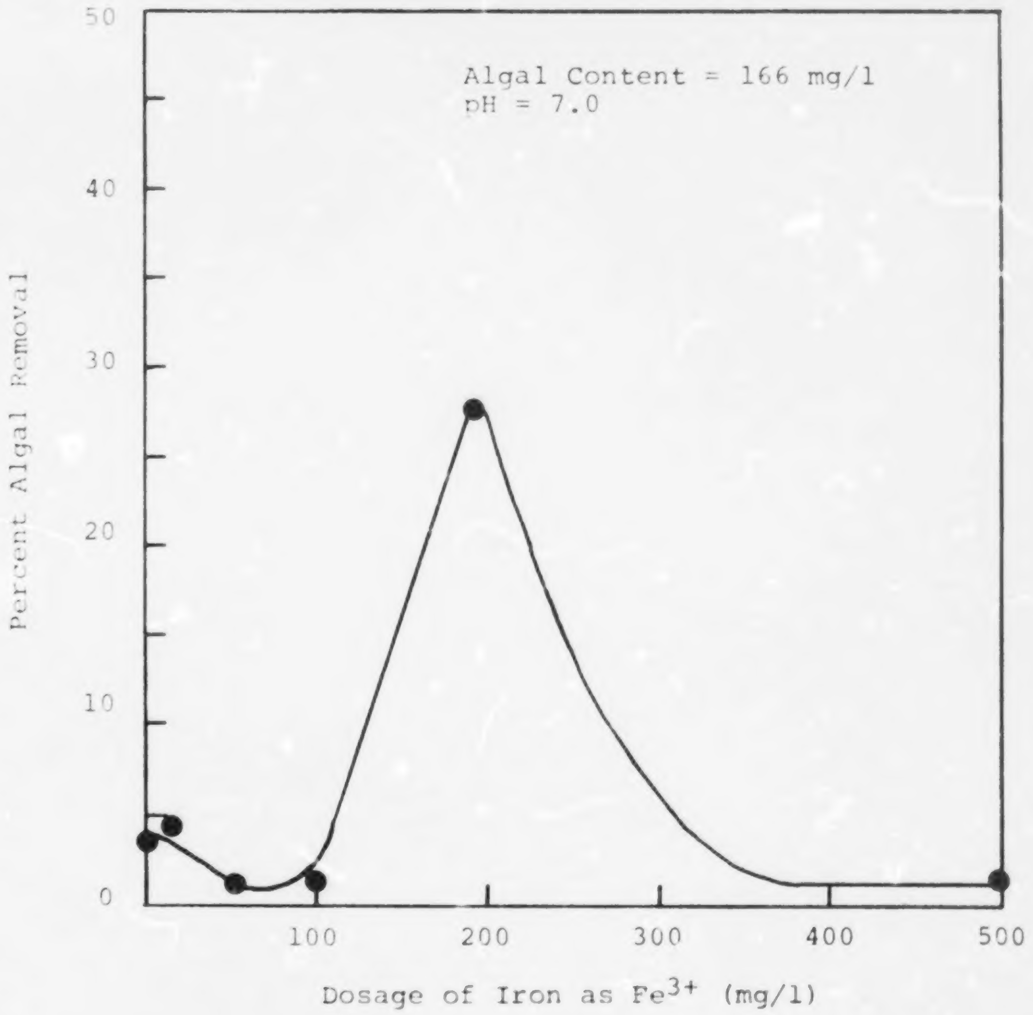

Figure 31 Algal Flocculation in a Harvested Culture with Varying Dosages of Iron at pH 7.0.

causing restabilization of the algal biocolloids (see Figure 32). These removals are markedly reduced from those illustrated in Figure 30. These data again support the theory that algal enmeshment in the iron-phosphorus complex was occurring. Although enmeshment of the algal cell in the phosphate precipitate appeared to be the major removal mechanism, some bridging probably occurred between algal cells as well as between the iron-phosphate precipitate and the algal cells.

However, flocculation did occur in the harvested culture indicating that the anionic polymer-ion bridge was capable of removing algae in suspension. The mechanism of algal flocculation appeared to be a combination of charge-neutralization and bridging or a combination coagulation-flocculation phenomenon.

Two final studies were performed to determine the effect of iron addition on algal flocculation with cationic and nonionic polymers. The initial jar test was conducted at a constant algal concentration of 170 mg/l, a constant pH of 7.0, a constant iron dosage of 50 mg/l as Fe^{3+}, and varying dosages of C-31 ranging from 0 to 15 mg/l. The results of this study, as shown in Figure 33, indicate an optimum C-31 dosage of 1.0 mg/l as compared to approximately 15 mg/l previously reported in Figure 25 for a similar system without iron additive. Apparently, the iron-phosphate complex is enmeshing the algae to such an extent that the polymer dosage required for destabilization of the algal suspensions is greatly reduced. However, the addition of the cationic polymer significantly enhances algal removal beyond that achieved with iron additives alone due to its ability to electrostatically attach to the algal biocolloid, thereby forming polymer bridges.

The last study conducted was intended to determine the effect of the optimum iron dosage on algal removal with a nonionic polymer. This jar test was conducted at a constant algal concentration of 168 mg/l, a constant pH of 7.0, a constant Fe^{3+} dosage of 50 mg/l, and varying dosages of N-11 from 0 to 20 mg/l. The results, shown in Figure 34, illustrate an optimum polymer dosage occurring at 0.5 mg/l. Algal removals observed were vastly improved over those obtained in algal suspensions flocculated with N-12 alone (see Figure 26). It is postulated that this improved algal removal is due to algal enmeshment in an iron-phosphate precipitate. However, the N-12 was observed to improve algal removal beyond that obtained

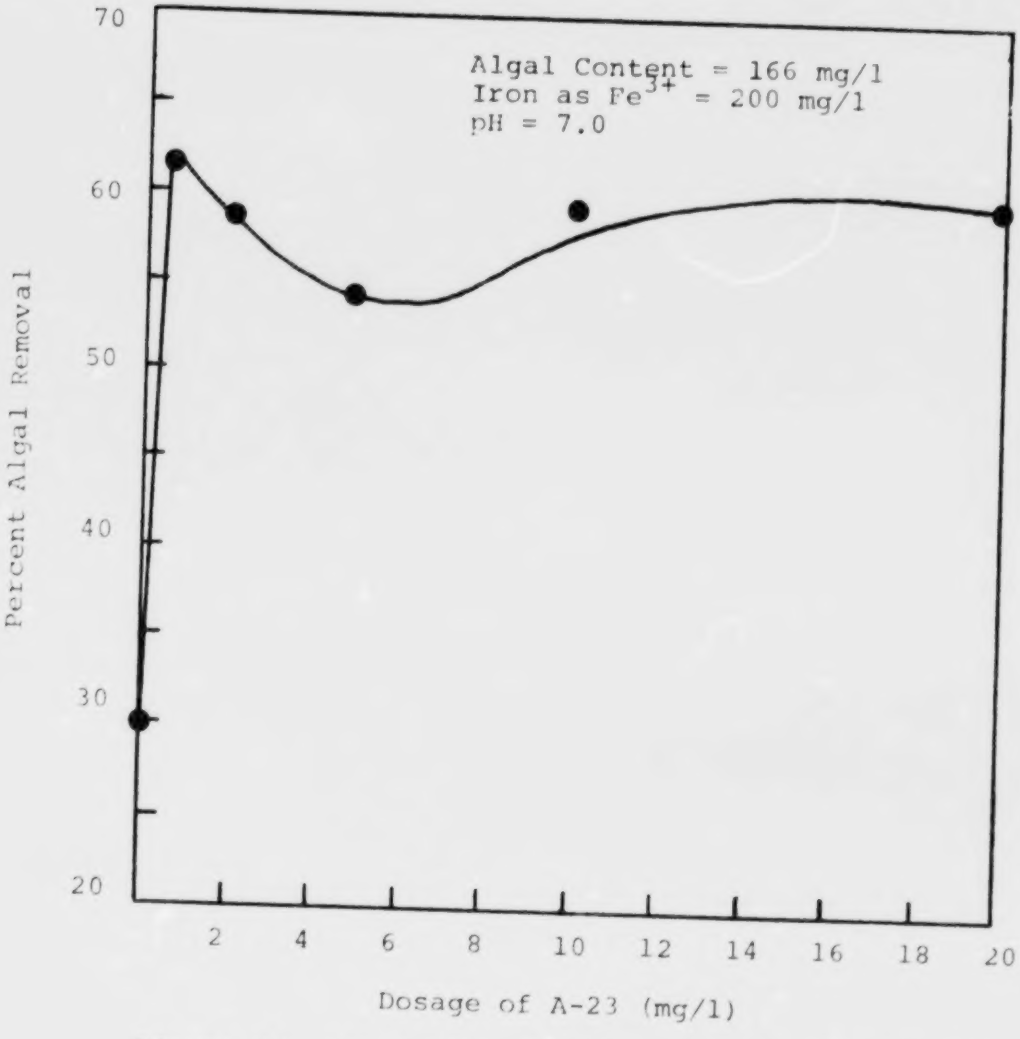

Figure 32 Algal Flocculation with the Optimum Iron Dosage (200 mg/l) in a Harvested Culture and Varying Dosages of Anionic Polymer A-23 at pH 7.0.

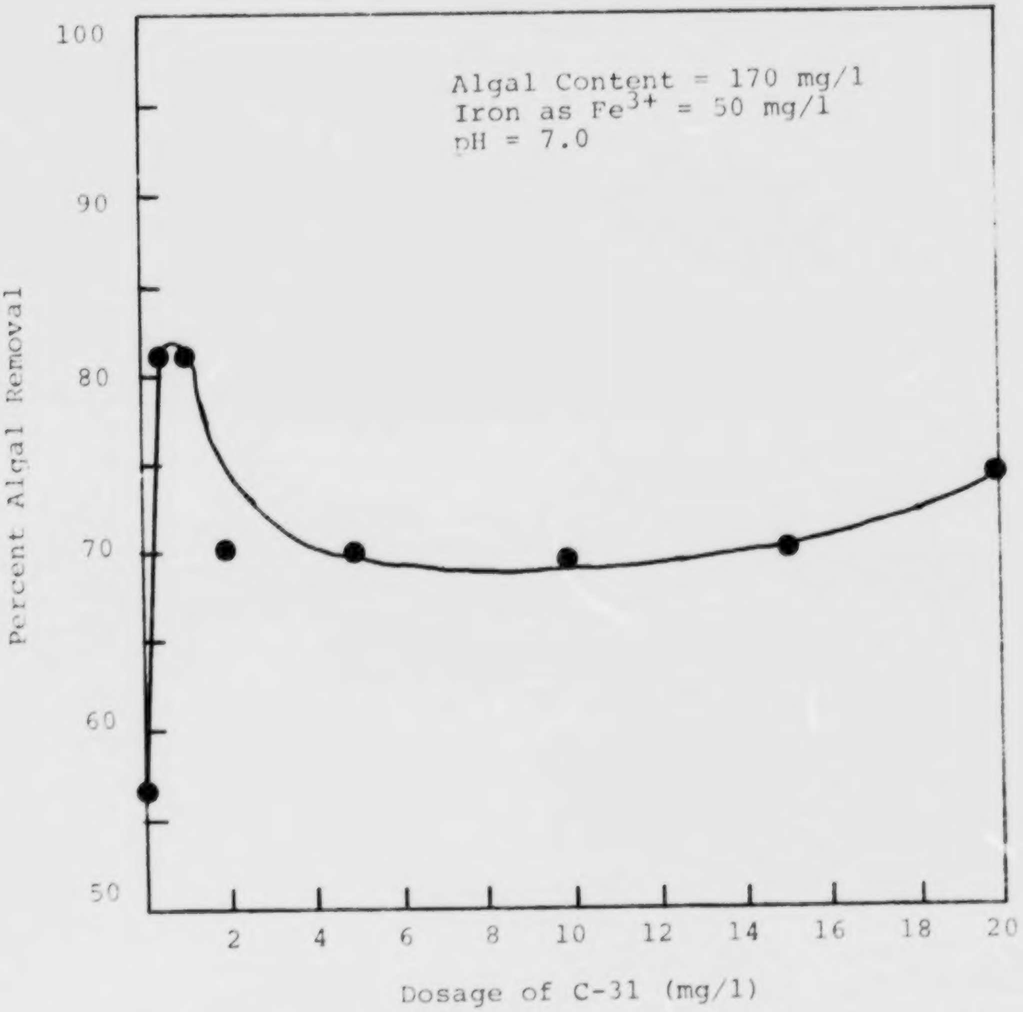

Figure 33 Algal Flocculation with the Optimum Iron Dosage (50 mg/l) and Varying Dosages of Cationic Polymer C-31 at pH 7.0.

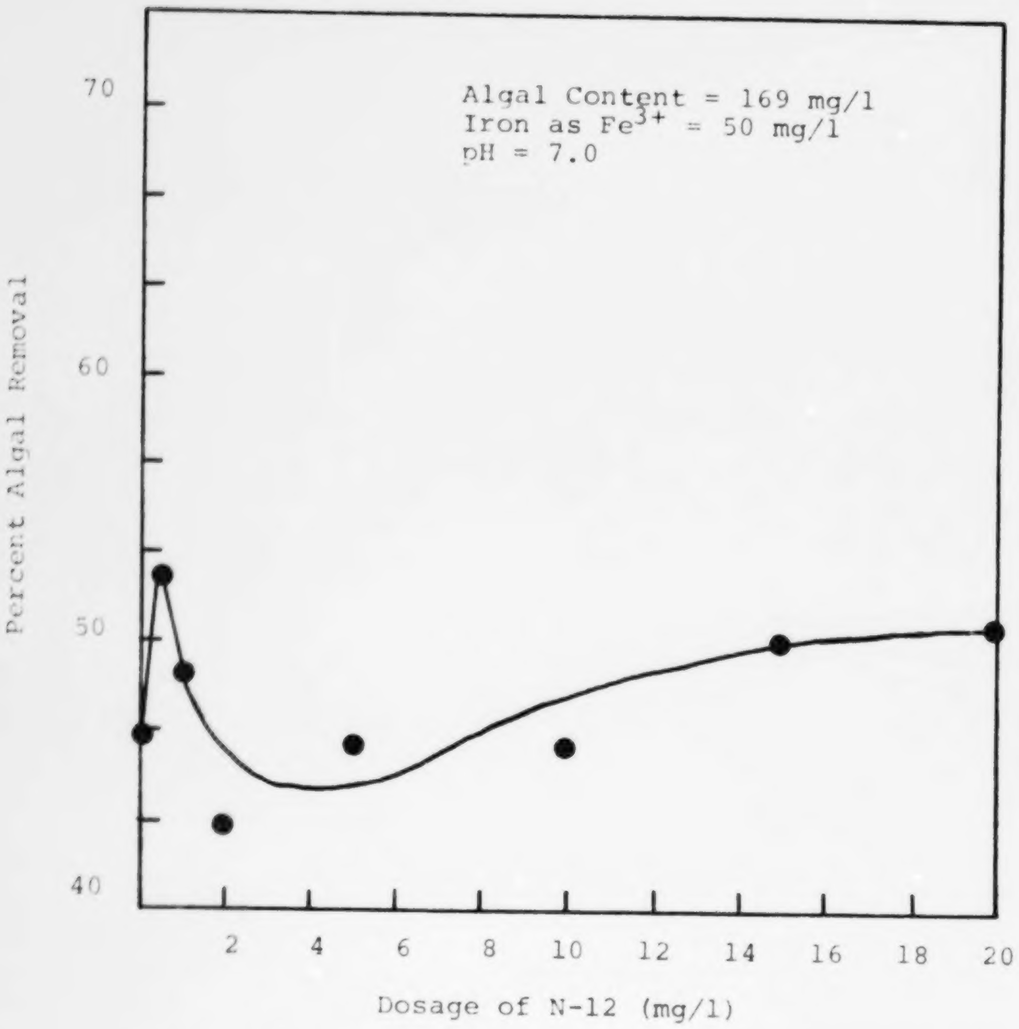

Figure 34 Algal Flocculation with the Optimum Iron Dosage (50 mg/l) and Varying Dosages of Nonionic Polymer N-12 at pH 7.0.

with iron addition only.

In summary, the mechanism of algal removal using nonionic and cationic polymers appears to be enmeshment in the phosphate precipitate being formed as a result of iron addition. Algal removals observed in cationic polymer-iron systems were considerably greater than those in nonionic polymer-iron systems since the cationic polymer electrostatically bridged between the algal cells, whereas the nonionic polymer had to rely on adsorption phenomenon for attachment to the biocolloid surface.

The conclusions of the chemical flocculation studies may be summarized as follows:

The optimum pH for algal agglutination with synthetic organic polyelectrolytes appeared to be in the range of pH 2.0 to 4.0, thereby implying that polymer flocculation mechanisms are governed by the algal cell surface charge.

Optimum dosages of all synthetic polymers at optimum pH levels appeared to be in the range of 0.5 to 5.0 mg/l with subsequent restabilization of the algal suspension at higher polymer dosages.

At neutral pH levels the cationic polyelectrolytes utilized appeared to effectively flocculate the algal suspension through a combination of polymer bridging and charge neutralization, whereas anionic and nonionic polyelectrolytes failed to efficiently flocculate the algae at pH 7.0 due to their inability to bridge the repulsive separation distance and chemically bond to the cell surface.

The addition of trivalent metal ions to algal suspensions flocculated with polyelectrolytes was observed to significantly enhance algal flocculation phenomena compared to algal removals obtained with either trivalent metal ion or polymer addition only. It is postulated that the trivalent metal ion enhances flocculation phenomena by reducing the algal cell surface charge (reducing biocolloid separation distances) and possibly serving as a bridge between the algal cell surface and the polyelectrolyte.

CONCLUSIONS

It appears that algae utilized in nutrient removal schemes for wastewater treatment may provide a potential food product if they can be efficiently harvested. Natural and induced flocculation techniques apparently may promote efficient algae-liquid separation at reasonable costs. The results of this investigation imply that algae harvesting by means of bioflocculation or chemically induced flocculation techniques can be accomplished in a very efficient manner under certain conditions. It, therefore, seems imperative that further research be focused on algal bioflocculation and chemically induced flocculation mechanisms on a pilot scale to develop pragmatic operational data which could be utilized to accomplish effective algal harvesting as a food source.

ACKNOWLEDGEMENT

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CRITICAL VARIABLES IN FOOD-ITEM POPULATION DYNAMICS IN A WASTE-WATER AQUACULTURE SYSTEM

by

Joseph E. Powers*

INTRODUCTION

Increases in human populations and the associated increases in domestic wastes have made it essential that beneficial methods of disposal of these wastes be developed. One suggestion was the addition of treated sewage to aquaculture ponds (Allen[1]). Nutrient-rich sewage waters added to an ecosystem would generally increase the primary production and lead to higher productivity in the system as a whole. But, in order for such a strategy to be effective in an aquaculture system, it is mandatory that the majority of the energy that results directly from the sewage addition be channelled into the species being cultured and not lost to other populations within the system. This implies that simple straight-chain food-webs would be most effective in aquaculture. Therefore, in managing aquaculture systems, it is imperative that there be an understanding of the key variables associated with the population dynamics of the major food organisms.

An aquaculture system fertilized by domestic sewage was constructed adjacent to Humboldt Bay, California (Allen, Conversano and Colwell[2]) for use in rearing salmon (Oncorhynchus kisutch) to the smolt age, i.e., the age at which they migrate to the ocean. Ultimately such a system will provide a return run of adult salmon to Humboldt Bay.**

The primary food organism which developed within this pond was the gammarid amphipod Anisogammarus confervicolus Stimson. Powers[3] reported on the structure and general results of a simulation model describing the dynamics of this amphipod population. That simulation model is now used to give evidence as to the nature of the critical variables affecting the dynamics of the amphipod population. This evidence is formulated into a possible management strategy and some generalizations concerning management of these types of aquaculture systems are made.

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**The ponds were constructed by the Conservation Board of the California Department of Fish and Game and are operated under National Sea Grant Funds, California State University, Humboldt Coherent Area Project, Northern California Coast.

THE AQUACULTURE SYSTEM

The Humboldt Bay aquaculture system consists of a 1.4 hectare pond located within the perimeter of a 55-acre oxidation pond. Tertiary-treated domestic sewage could be introduced from the oxidation pond to be mixed with sea water from Humboldt Bay.

Major animal populations existing in the pond (other than the salmon and amphipods) were two species of fish: sticklebacks (Gasterosteus aculeatus) and topsmelt (Atherinops affinis). These fish entered as larval or egg forms through a metal screening device which strained the inlet water from Humboldt Bay. Holes in the device were six millimeters in diameter. These apertures were the means by which the amphipods gained access to the pond, as well.

THE SIMULATION MODEL

The model consists of a system of difference equations that are updated each of 22 time periods. Each period corresponds to three days of actual time. The amphipod population was divided into age classes and at each time period the vital statistics of ration size, growth, predation by fish populations and natural mortality, i.e., mortality other than predation (assumed to be the result of the direct effect of temperature and salinity) were computed for each age class. Inputs into the model consisted of initial population estimates for the fishes and amphipods, temperature and salinity measurements, predation rates of fish on the amphipods, amphipod reproduction rates and particulate organic matter (POM) measurements, all of which were taken directly from studies of the aquaculture pond (POM measurement method was that of Lovegrove[4]).

The initial simulation used these inputs exactly as they were measured in the pond. Subsequent runs were made with perturbations of most of the inputs in order to discern the key relationships.

RESULTS

Most of the amphipods that entered the pond through the Humboldt Bay inlet were adult forms (Figure 1) and, therefore of too large a size to be utilized by the small fingerling salmon. In order to maintain a stable food-item population in the system, the population must be allowed the time to develop. The importance of this lag time and its approximate length can be observed in Figure 2. This figure actually displays two lag times: 1) the lag between conversion of the sewage nutrients to POM, and 2) the subsequent conversion of POM to amphipod biomass. The pond was filled with sewage water and sea water seven days before the initial time used in the simulation. Approximately 30 days lag time was needed for POM build-up and 45 days for amphipod build-up.

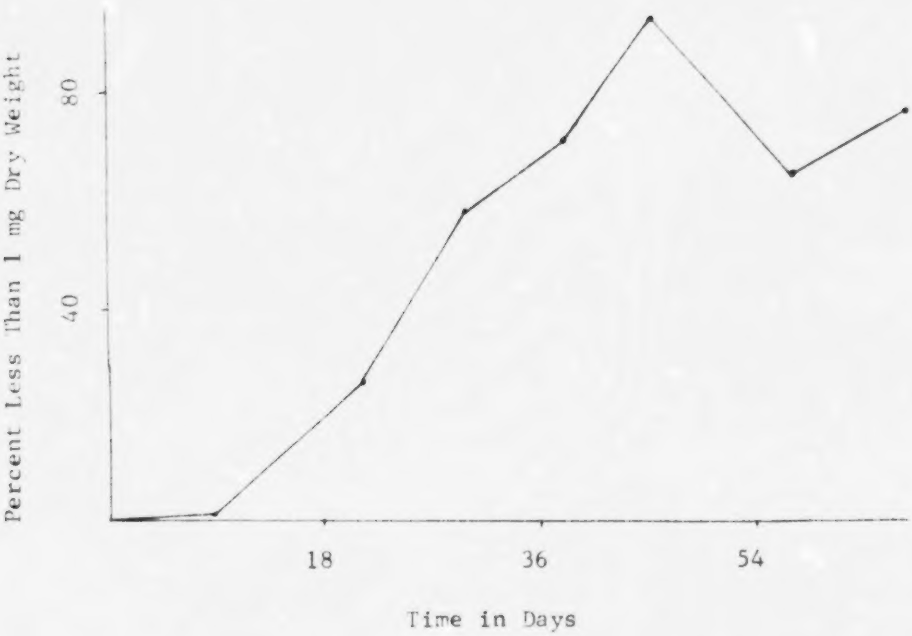

Figure 1. Changes in Amphipod Size Distribution with Time.

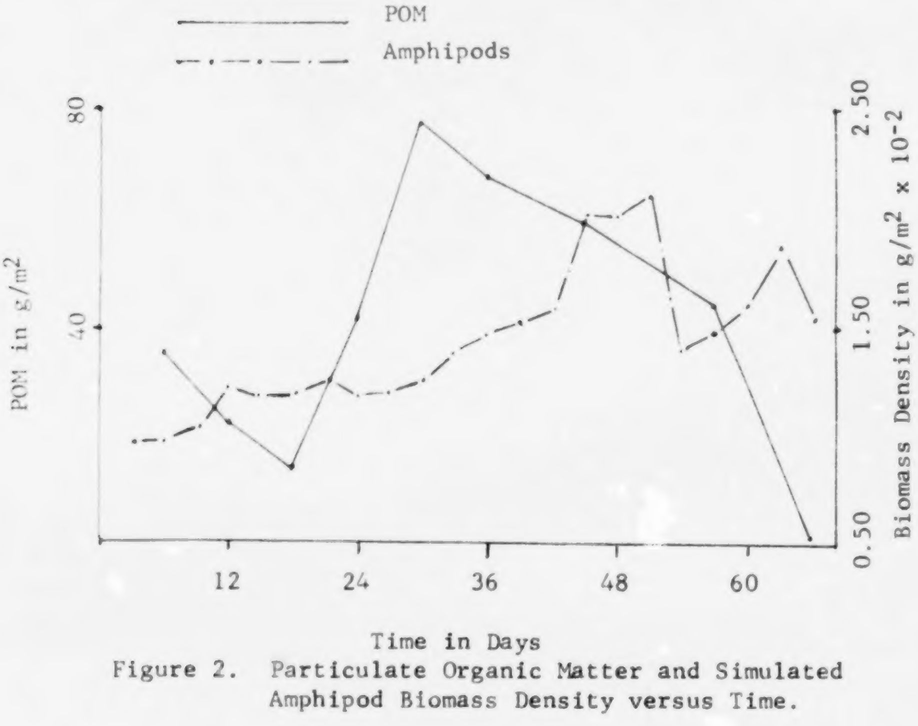

Figure 2. Particulate Organic Matter and Simulated Amphipod Biomass Density versus Time.

Table 1. Effects of Salinity on Amphipod Growth Rate

Salinity (ppt)		20	24	16
	Age (days)	Ave Weight (mg dry)	Ave Weight (mg dry)	Ave Weight (mg dry)
	3	0.376	0.377	0.373
	6	0.468	0.471	0.462
	9	0.581	0.587	0.569
	12	0.718	0.728	0.697
Average Growth Rate (mg dry/day)		0.038	0.039	0.036

Table 2. Effects of Density on Amphipod Growth Rate

Amphipods/m^2		188.27	782.23
	Age (days)	Ave Weight (mg dry)	Ave Weight (mg dry)
	3	0.376	0.311
	6	0.469	0.384
	9	0.583	0.476
	12	0.722	0.588
	15	0.889	0.721
Average Growth Rate (mg dry/day)		0.043	0.034

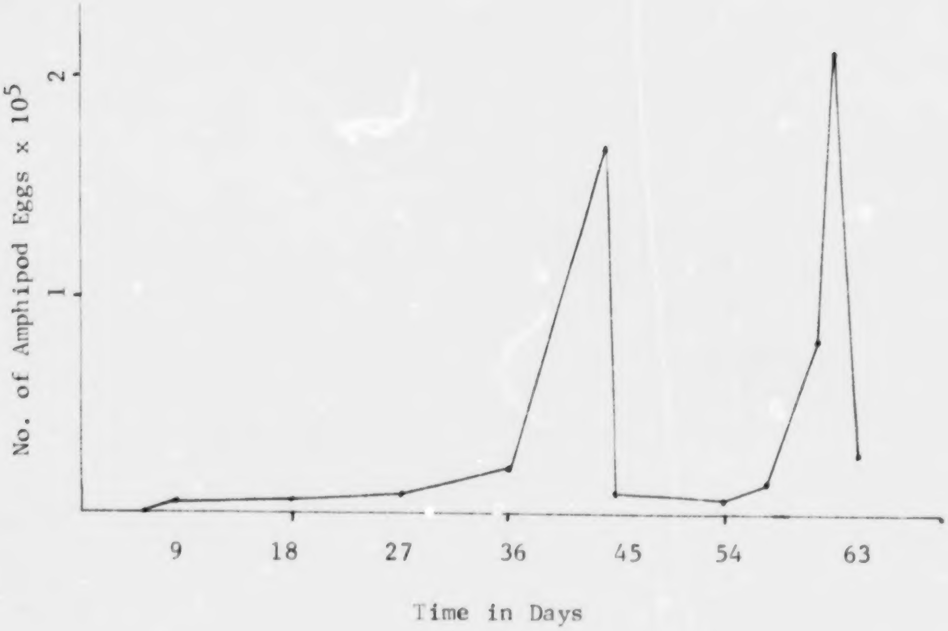

Figure 3. Simulated Egg Production versus Time

A decrease in the lag time would be a desirable goal in an aquaculture system. This could be attained by starting with an amphipod population composed primarily of young animals and/or by having an increased growth rate of the amphipods. Manipulations of several of the system variables could produce such changes in growth rate.

Salinity changes caused flux in the growth. Results from computer runs at three different salinities on day 24 of the simulation are shown in Table 1. The average growth rate of 15-day-old smphipods appeared to be greatest at a salinity of 24 parts per thousand (ppt). Similarly, the effects of population density on day 45 of the simulation are shown in Table 2. Quadrupling the amphipod density resulted in a 26.4 percent decrease in the growth rate over the first 12 days of life.

Another cause for this lag in biomass production is the reproductive process (Figure 3). Total reproduction did not rise appreciably until day 36. This rapid increase was caused by growth into reproductive age of the amphipods which, themselves, were produced in the pond. It was at this stage that the size distribution of the pond began to stabilize.

DISCUSSION

A major concern in managing an aquaculture system is that of establishing an adequate food source for the species being cultured. In the Humboldt Bay salmon culture project, it appears that this can be achieved most effectively by maintining a brood stock of amphipods and by controlling the salinity.

Maintenance of a brood stock would mean assuring the survival of a sufficient number of amphipods from one culture session to the next, i.e., the pond should not be completely drained allowing some habitat in which the amphipods could reside. Also, the pond should be refilled as soon as possible after the partial draining to further minimize mortality.

Salinity manipulations could be used to maximize amphipod growth rate. If the initial salinity was kept at 24 ppt, the amphipods would grow fast and quickly establish the population. This salinity (24 ppt) was shown to be optimum from the laboratory studies using in fitting data to equations in the simulation model[5]. Then salinity could be lowered (the proportion of freshwater could be increased) to meet the physiological needs of the salmon. Temperature would affect growth rate as well, but it is unlikely that temperature control would be economically feasible.

A large number of sticklebacks and topsmelt entered the aquaculture system and grew to a size that was capable of exploiting the amphipods. This introduced the element of interspecific competition to the salmon.

This aspect should be eliminated from the system. Since the incoming water cannot be screened any more finely (or else the amphipods could not enter) the time of the year the ponds are filled should not coincide with the times in which large numbers of eggs and larval fish appear in Humboldt Bay.

CONCLUSION

Sewage water can bring added productivity to an aquaculture system, and by judicious management that productivity can be shunted into the desired species. The food-item must be allowed to develop a sufficient population. The time needed for this development can be decreased by controlling system variables at the optimal levels for the food-item. Once the food-item population is somewhat stable and able to withstand environmental pressure, the system variables should be converted to levels optimal for the species being cultured. Species which do not contribute directly to the cultured species should be eliminated from the system entirely.

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THE FEASIBILITY OF PENAEID SHRIMP CULTURE IN BRACKISH PONDS RECEIVING TREATED SEWAGE EFFLUENT*

William L. Rickards**

INTRODUCTION

The study described herein was one part of an extensive investigation of the ecological structure and functioning of brackish water pond systems receiving treated sewage effluent. The studies were conducted at the University of North Carolina Institute of Marine Sciences in Morehead City, N. C. from 1969 through 1972. Details concerning the results of the four-year program were presented by Odum and Chestnut,[1] Kuenzler and Chestnut,[2,3] and Kuenzler, Chestnut and Weiss[4] in project annual reports.

Experimental facilities for the program consisted of six 0.1 acre ponds, three of which received brackish tidal creek water plus treated sewage effluent while the remaining three ponds received only brackish water and served as control units.

The long-range goals of the overall sewage study program included the development of methods of aquaculture which would utilize the effluent of sewage treatment plants as well as providing a screening device for organisms with the ability to withstand the environmental fluctuations which occur in ponds receiving the effluent.

During the two previous years of the pond project, penaeid shrimp had been stocked in both the control ponds and the sewage ponds. The results of these stocking attempts were described by Beeston[5,6]. In both cases, the shrimp survived and grew in the control ("C") ponds but failed to survive in the sewage ("P") ponds. At the time, it was the opinion of project personnel that failure of the shrimp to survive in the "P" ponds was due to the very low levels of dissolved oxygen in the water at night (Smith[7]) as well as rather wide diurnal pH fluctuations (Laughinghouse and Kuenzler[8]).

Despite the failure of penaeid shrimp to survive, aquacultural interest in them because of their economic value prompted the study described below. The objectives of this study were to modify the oxygen and pH regimes in one of the "P" ponds and to subsequently stock the pond with penaeid shrimp to determine whether or not factors in addition to oxygen and/or pH had been responsible for the previous shrimp mortalities.

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**University of North Carolina Sea Grant Program, 1235 Burlington Laboratories, North Carolina State University, Raleigh, N. C. 27607

I. HABITAT IMPROVEMENT STUDY

In an attempt to alter the "P" pond habitat so that penaeid shrimp would be able to survive, P-2 was selected for a study in which the pond was aerated. It was hoped that a relatively small amount of aeration would alleviate the extremely low dissolved oxygen encountered in the early morning hours by other investigators (Smith[7]). Since penaeid shrimp generally require dissolved oxygen levels of at least 2.0 ppm, 3.0 ppm was considered a desirable level to attempt to maintain in P-2.

METHODS

Prior to aeration, pond maintenance procedures involved routine daily measurement of dissolved oxygen, temperature, and pH in the evening and early morning. In addition, a diurnal dissolved oxygen curve was determined on 20-21 June, 1971. On 14 July 1971, aeration of the pond was begun by means of a 3/4 horsepower, oil-less compressor and 200 feet of perforated plastic air hose. The hose was arranged in a four-leaf clover pattern so that all areas of the pond received aeration. During the period 14-22 July, dissolved oxygen, pH, and temperature fluctuations were monitored. Temperature and pH were measured by recording instrumentation at the pond. Dissolved oxygen was determined by Winkler titration of samples fixed at the pond and returned to the laboratory.

RESULTS AND DISCUSSION

Prior to aeration, the dissolved oxygen varied diurnally from 0.0 mg/l to about 13.5 mg/l as shown in Figure 1. Values plotted in Figure 1 are averages of triplicate measurements taken at different depths and locations in the pond.

Following aeration, fluctuations in the diurnal curve had moderated (Figure 1). Neither the daytime peak nor the early morning low point reached those experienced without the aeration. Of greatest interest was the maintenance of an average minimum dissolved oxygen value of 3.4 mg/l over the seven day period of measurement.

In addition, the pH which had at times varied from 7.5 to nearly 10.0 on a diurnal basis was now being maintained within a much narrower range, 7.6 to 8.3 Water temperatures did not differ noticeably between the two periods being compared.

Use of the compressor and perforated air line did not stir up the bottom sediments. This could have been a problem since increased turbidity in the system would have resulted in lower rates of photosynthesis by the phytoplankton possibly pushing the system past the point of compensation where production balances respiration. This would not be desirable since phytoplankton production is the basis

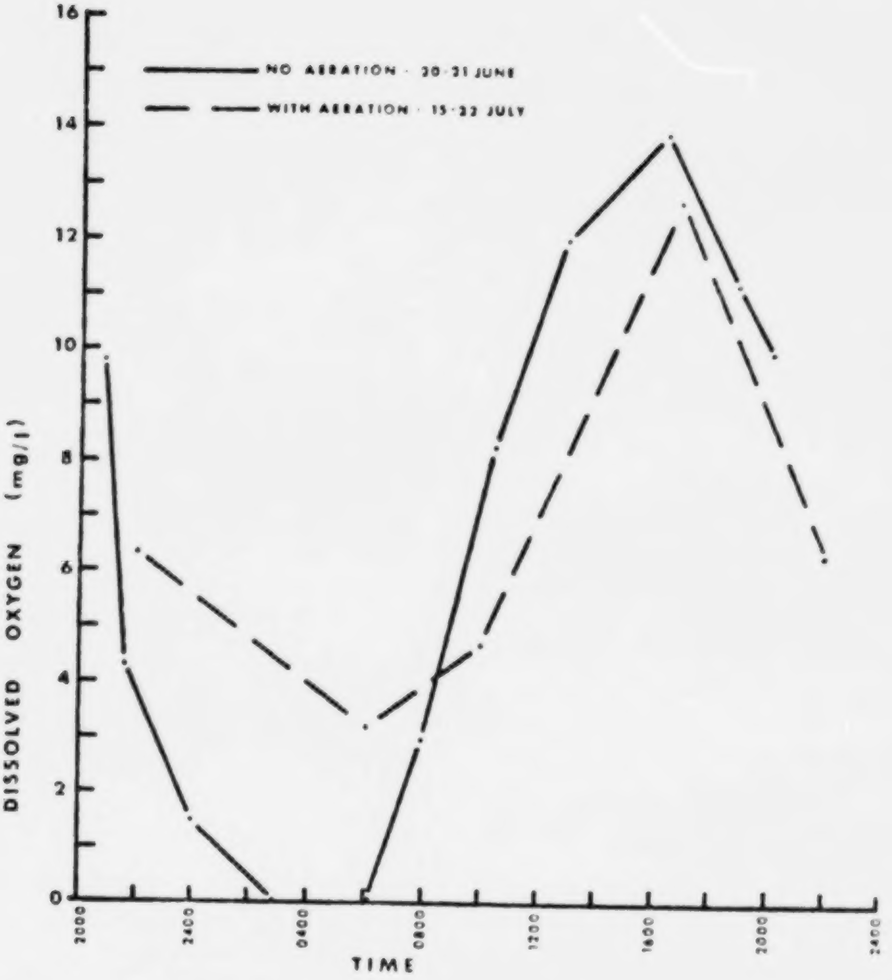

Figure 1. The average dissolved oxygen in milligrams per liter on a diurnal basis for Pond P-2 before and during aeration.

for the food chains being investigated as sources of aquacultural products in systems receiving treated sewage effluent.

CONCLUSIONS

Aeration of pond P-2 by the means employed maintained dissolved oxygen well above 3.0 mg/l which had been accepted as the minimum desirable level.

Aeration had no detectable effect on temperature, but diurnal fluctuations in pH were moderated as had been desired at the beginning of the study period.

As a result of the modification of the pond environment into one which was more favorable for penaeid shrimp, it was decided to undertake studies to determine whether or not shrimp could now survive and grow in the "P" ponds.

II. SHRIMP SURVIVAL

The objective of this aspect of the study was to determine whether or not penaeid shrimp could survive in the modified environment of pond P-2. If survivors were found, growth would then be measured.

METHODS

On 20 August 1971, juvenile white shrimp (Penaeus setiferus) were seined from Hoop Hole Creek, a tidal slough on the sound side of Bogue Banks near Atlantic Beach, N. C. The shrimp were transported to the laboratory and transferred to a holding pen through which water from Bogue Sound circulated. The shrimp were held overnight and were released into ponds C-2 and P-2 the next morning. Thus, on 21 August, C-2 received 221 juveniles and P-2 received 211 juveniles.

Additional juvenile P. setiferus were added to the same ponds on 13 September bringing the total numbers of shrimp to 321 in C-2 and 287 in P-2. No attempt was made to stock the ponds to carrying capacity. A sample of 35 of the juveniles were preserved in 10% formalin for weight and length determinations.

On 10 November seine hauls were made in each pond stocked. Sampling recovered 60 shrimp from C-2 and 33 shrimp from P-2. Weight and length were determined for each shrimp.

Because shrimp were released into the ponds on two occasions, 21 August and 13 September, those shrimp recovered on 10 November were in the ponds for either 82 or 59 days. Data discussed in the following sections are based on an average stocking data of 1 September giving an average duration in the ponds of 70 days.

RESULTS AND DISCUSSION

Figure 2 shows the average initial and final weights of the shrimp recovered from P-2 and C-2. Whereas shrimp in both ponds were stocked at the same average weight (1.4 grams) those in P-2 grew to a slightly greater average weight (14.8 grams) than did those in C-2 (13.45 grams). Average shrimp lengths were also somewhat larger for shrimp from P-2 than for those from C-2 (Table 1), 121.5 mm as compared to 113.3 mm.

The increase in weight of shrimp in P-2 was 13.4 grams in approximately 70 days or 0.97 mm in length per day. Such growth compares favorably to that of shrimp in natural populations in North Carolina (McCoy[9], McCoy and Brown[10], Williams[11]). Thus, it would appear that the "P" pond environment may be made suitable for growing penaeid shrimp simply by aeration of the water.

Differences in the numbers of shrimp recovered from P-2 and C-2 may have been due to several reasons. However, the most likely reason would be the difference in bottom type between the two ponds. C-2 has a fairly firm bottom in which seining is much easier than in P-2 which has a very soft, muddy bottom into which the shrimp can burrow and which makes seining very difficult.

In addition, mortality of the stocked shrimp may have been greater in P-2 than in C-2. This could not be verified since it was impossible to recover all of the shrimp in either of the ponds. The presence of numerous blue crabs in P-2 could have resulted in greater losses of shrimp in that pond than in C-2 in which fewer blue crabs were seen.

Also shown in Figure 2 are the changes in heads-on count (number of shrimp per pound) which occurred during the study. When stocked, the juveniles were approximately 300 count heads-on, and they were approximately 30 count in P-2 and 50 count in C-2 on 10 November. Shrimp of 30 count weight are well within a marketable size range. Thus, shrimp of commercial size were produced in the pond receiving treated sewage effluent.

Supplementary feeding was not employed during the study, and it is assumed that the shrimp were feeding on the numerous small organisms and accumulated detritus in the ponds.

CONCLUSIONS

Penaeid shrimp were able to survive and grow in the aerated "P" pond. It is now logical to proceed with studies of penaeid shrimp food chains utilizing treated domestic effluent and production dynamics in aquacultural applications.

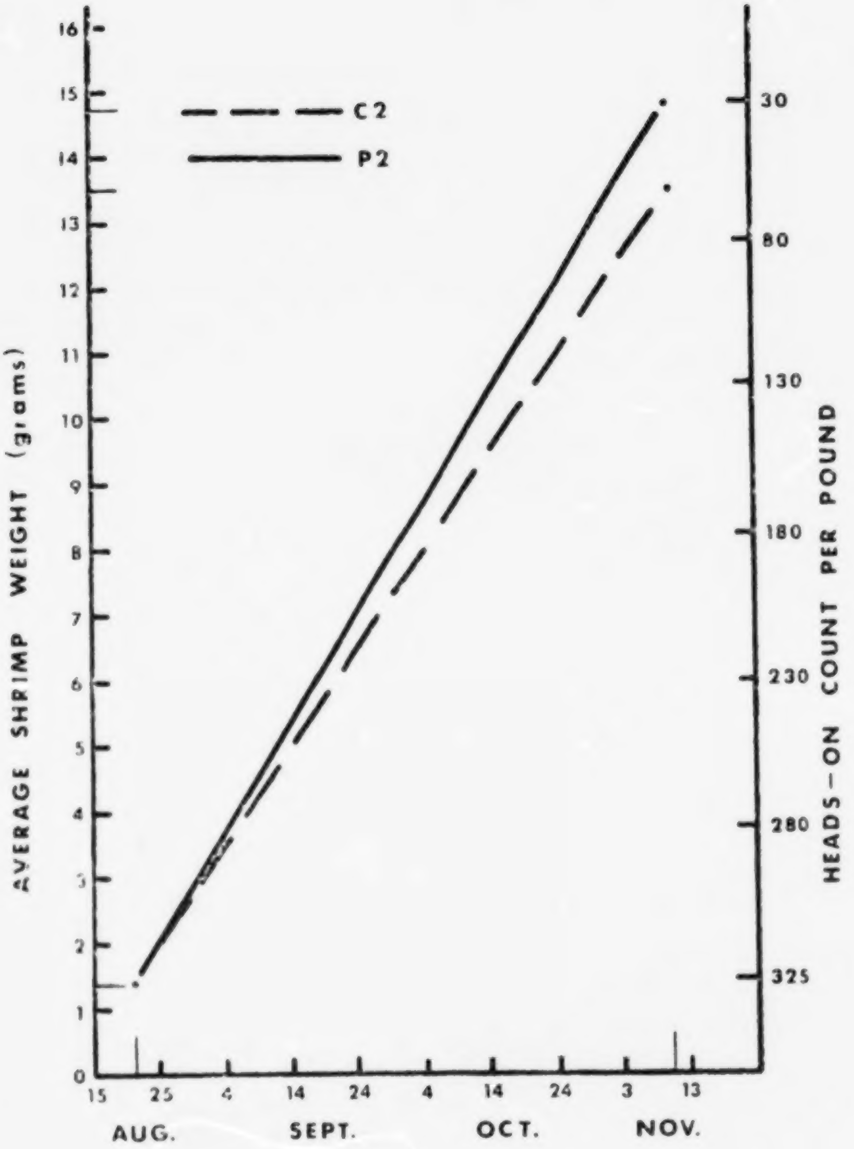

Figure 2. Changes in the average weight and heads-on count (number of shrimp per pound) during the study period for shrimp stocked in ponds P-2 and C-2.

Table I. Data obtained from shrimp stocked in ponds P-2 and C-2. Shrimp lengths were measured from the tip of the telson to the base of the rostrum. Data ranges are in parentheses.

	SHRIMP IN POND P-2	SHRIMP IN POND C-2
Number stocked	287	321
Average No. days in pond	70 (59-82)	70 (59-82)
Average initial weight (grams)	1.40 (0.4-3.1)	1.40 (0.4-3.1)
Average initial length (mm)	57 (36-75)	57 (36-75)
Number sampled 10 Nov.	33	60
Average final weight (grams)	14.77 (5.0-21.0)	13.45 (4.0-21.0)
Average final length (mm)	122 (92-139)	113 (84-130)

Shrimp growth in the "P" ponds approximated that found in natural populations of penaeid shrimp in North Carolina. Thus, there appeared to be no detrimental effects on growth resulting from the extremely eutrophic environment employed.

It appears that such eutrophic environments may be beneficially employed as grow-out ponds in shrimp culture operations since shrimp of marketable size were produced. In addition, it may be possible to obtain three crops from such grow-out facilities in North Carolina depending on the duration of favorable water temperatures in a particular year.

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STANDING CROPS OF BENTHIC FAUNA IN MARINE AQUACULTURE PONDS USING RECLAIMED WATER

by

Thomas R. Sharp*

INTRODUCTION

In a time of worldwide shortages of food and energy, it is essential that new methods of human food production be developed. One possible method is to produce fish for human consumption from domestic wastewater (Allen[1]). An important link between wastewater nutrients and fish production is the benthic fauna, a basic food for most fish (Darnell[2]). The placing into operation of two experimental aquaculture ponds in July 1971 located within the confines of a city wastewater treatment plant next to the tidal flats of North Humboldt Bay (Allen and Dennis[3], Fig. 1) provided the opportunity for studying the effects of treated domestic wastewater on estuarine benthic fauna.

The primary purpose of this study was to determine the differences in benthic standing crops between the fertilized pond and the unfertilized pond. North Pond was managed as a static estuarine-sewage effluent mixture. The South Pond was an estuarine pond only, and was tidally flushed every 2-3 weeks (Allen and Dennis[3], Figures 1-3).

To determine a desirable substrate for fish food production in future aquaculture ponds, the benthic standing crops on four available substrates were studied; substrates included mud, sand(Hookton soil), river-run gravel, and oyster shell. Pens to exclude fish were used to determine the effect fish predation had on benthic standing crops. Since this study began with the initial filling of the ponds, the colonization of the ponds was studied for comparisons with conditioned pond bottoms in future experiments.

METHODS AND MATERIALS

Temporal Changes in Benthos

In order to study fluctuations in benthic animal nutrients over time, a transect technique was employed. A transect

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line was marked by a rope stretched between the headgate and the northeast corner of each pond. This line was located over areas of mud substrate only (Sharp[4]). Monthly, beginning September 1971, five core samples were taken at 6 meter intervals along the transect line of each pond. Ten samples were taken on each sampling date for a total of 50 samples over the course of the study. The time of the study corresponds to Pond Experiment 1B (Allen and Dennis[3], Table 2). Thus the early stages of succession were not covered.

Benthos was sampled using a Carrin core sampler (Carrin[5]) having a diameter of 4 inches (surface area of about 80 cm^2). Each sample was washed through a 0.457 mm Tyler screen and the part remaining preserved in 10% formalin solution. The organisms were sorted to species and counted. The specimens of a species in a sample were dried at 100°C for 24 hours (Hanks[6]) and weighed on an analytic balance.

Standing Crop

In order to study the standing crop of benthos at the end of the rearing experiment, an "in-place" sampling technique was used. An in-place basket sampler similar to that of Hanks[6], with modifications to accommodate the different substrates and changes in water level was employed (Figure 1). The sampler consisted of a 2-quart polyethelyne basket with a surface area of 200 cm^2 and a volume of 1600 cc. Triangular cut-outs covered by 0.457 mm Nitex nylon were made into the sides of each sampler to allow for water circulation which might occur in the substrates, especially in shell. A brick and float were attached by two lengths of nylon twine. The brick prevented the float twine from interfering with the substrate surface.

"Exclusion pens" enclosing an area of 4 m^2 were built on each of four substrates within each pond (Sharp[4]). On July 13, 1971 the in-place basket samplers were installed as follows. At each station (substrate within a pond) six samplers were filled with the appropriate substrate and placed so that the surface of the sampler was flush with the surface of the pond bottom. Three of the samplers were put inside the exclusion pen and three outside the pen (Sharp[4]). Thus, with four substrates in each pond, and two ponds, a total of 48 samples were installed (Figure 2).

The ponds were initially filled July 21, 1971 and finally drained on January 15 and 22, 1972. The samplers were withdrawn from North Pond December 13, 1971 and from South

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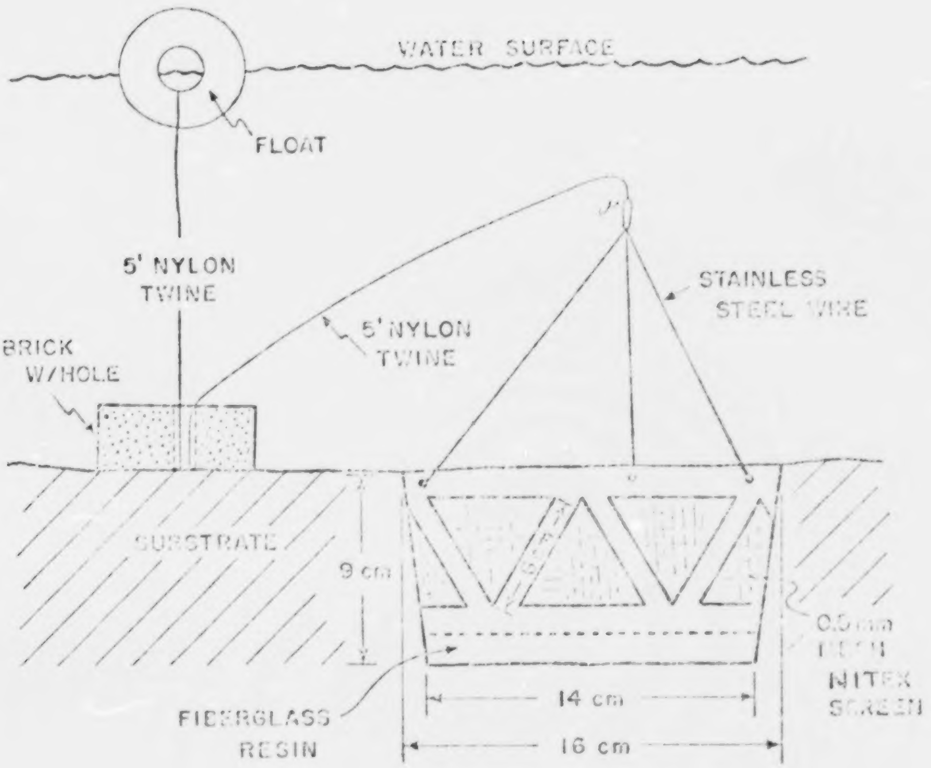
WATER SURFACE
FLOAT
5' NYLON TWINE
5' NYLON TWINE
STAINLESS STEEL WIRE
BRICK W/HOLE
SUBSTRATE
9 cm
0.5 mm MESH NITEX SCREEN
14 cm
16 cm
FIBERGLASS RESIN

Figure 1. In-place Basket Sampler used for Substrate study (top surface area, 200 cm^2; volume 1600 cc).

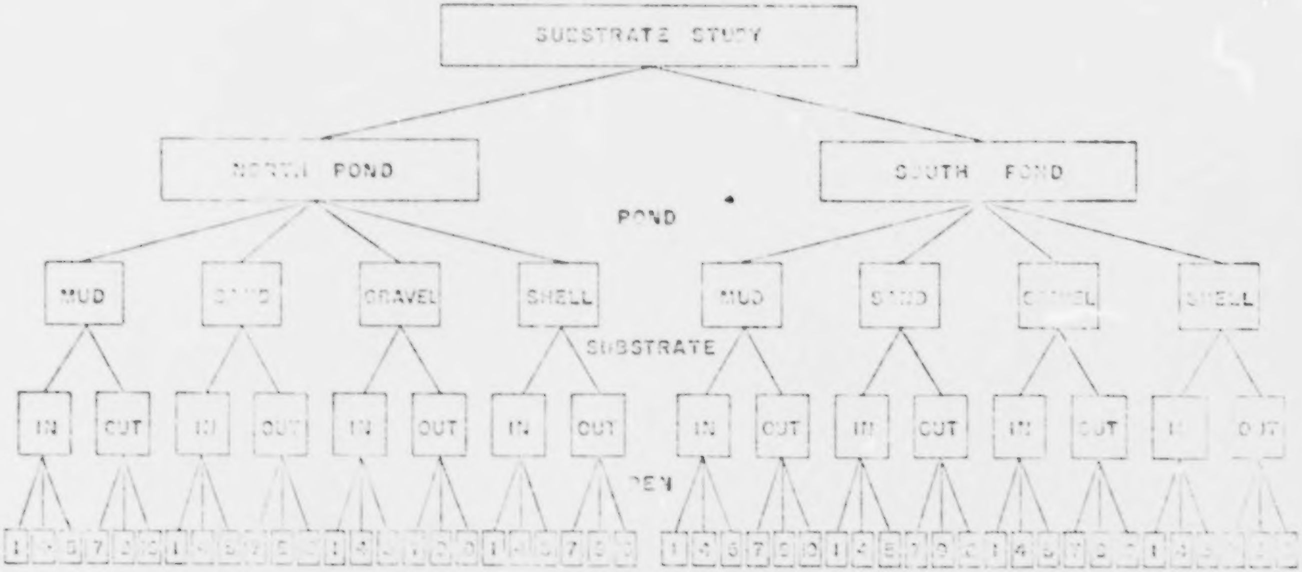
SUBSTRATE STUDY
NORTH POND
SOUTH POND
POND
MUD
SAND
GRAVEL
SHELL
SUBSTRATE
IN
OUT
PEN

Figure 2. Flow diagram of the substrate study.

Pond December 20, 1971, before either pond was drained.

Benthos and sediments in the basket samplers were processed in the same manner as that used for the transect samples with the following exceptions:

1. Mud, sand, and gravel samples underwent a sugar floatation technique (Anderson[7]) to eliminate the large volumes of substrate remaining after seiving.
2. Due to the high numbers of organisms in North Pond, all North Pond samples were divided in half using a Folsom Plankton Splitter and the results doubled.
3. For weighing, the three replicate samples on each test area were pooled and the results averaged.

METHODS OF ANALYSIS

A three-way analysis of variance (Sokal and Rohlf[8]) was employed to analyze the number of benthic animals only taken in basket samplers. The analysis was to test which treatment (pond, substrate, or exclusion pen)was significant for each of the major species found in the study. A standard logarithmic transformation (100 log [X+1] where X is the number of animals in each sample) was used to make the data more homogenous. Calculated F values for each treatment were compared with tabulated F values for a significant level of p=.05. If the F value was larger than the tabulated value, the treatment was considered significant.

A Student-Newman-Kreuls Test for multiple comparisons among means based on equal sample size (Sokal and Rohlf[8]) was used to determine significant factors within treatments. If the treatment being considered was substrate, the factors considered would be mud, sand, gravel and shell.

Numbers/M^2 and biomass(dry weight/M^2) were calculated from both transect samples and in-place basket samples. Wet weights/M^2 were calculated from dry weight data using conversion formulae (Thorsen[9]). Total numbers/M^2 and wet weights/M^2 were calculated for ponds, substrates and exclusion pens, using in-place basket sample data and for each sampling period using transect sample data. The basket sample totals were compared with other studies.

RESULTS

Organisms Present

The organisms found in the ponds included two species of polychaete, *Capitella capitata* Johnson (Capitellidae), and

Polydora ciliata Fabricus (Spionidae); and two species of gammarid amphipod, *Anisogammarus confervicolus* Stimpson, and *Corophium spinicorne* Stimpson (Table 1). *Daphnia spp* shells were found in large numbers but were considered artifacts from the oxidation pond. Bryozoans were not quantified. Great numbers of dipteran insect pupae casts were found but not considered part of the benthos. Other species occurred but in low numbers.

Changes in Standing Crops with Time

Both polychaete populations increased to November or December and then declined for both ponds (Table 2). North Pond (with sewage effluent) maintained generally higher populations with one notable exception: *C. capitata* standing crops peaked at the same level in both ponds (62.8 g/m^2 wet weight in North Pond compared with 62.4 g/m^2 wet weight in South Pond).

The two amphipod populations varied inversely between ponds (Table 2). *A. confervicolus* populations showed a continuous buildup in North Pond to the end of the experimental period with very few organisms occurring in South Pond. *C. spinicorne*, on the other hand, started with large populations in South Pond which continually decreased to total disappearance by December. Very few *C. spinicorne* were found in North Pond transect samples.

An unexpected relationship between *A. confervicolus* and *P. ciliata* was noted. Both numbers and weights showed high positive correlations between these two species (0.42 and 0.52 respectively). Both species also showed increases in number with decreases in surface temperature and salinity (Allen and Dennis[3]). The correlation could be by chance and should be investigated further.

The total disappearance of the benthos on South Pond mud by December remains unexplained. I sampled water properties in January and found low dissolved oxygen directly over the South Pond mud substrate only (Table 3). This strongly suggested a non-uniform mixing resulting in low DO's as a possible cause for the disappearnace. Populations on other substrates in South Pond seemed uneffected.

Effects of Fish on Standing Crop

No significant difference existed between samples taken inside and samples taken outside of the fish exclusion pens in this study. The lack of survival of salmonids in the initial experiments (Exp. IA, Allen and Dennis[3])

Table 1. Frequency of occurrence of benthic animals taken by two methods of sampling of fauna developed between July 1971 and January 1972 in two marine aquaculture ponds, one fertilized with treated wastewater.

Kind of Animal		Frequency of Occurrence	
Group	Species	Transect Sampling (N=50)	Basket Sampling (N=48)
Polychaete worms	*Capitella capitata*	97.9	83.3
	Polydora ciliata	47.9	83.3
Gammarid amphipod	*Anisogammerus confervicolus*	43.7	79.2
	Corophium spinicorne	33.3	52.1
Bryozoans		undetermined, but common	
Isopod (Flabellifera)		2.1	4.2
Barnacle	*Balanus spp.*		4.2
Mollusk	*Lyonsia californica*		4.2
Fish	*Gasterosteus aculeatus* (stickleback)		4.2

Table 2. Monthly average number and wet weight in grams per square meter of four major species of benthic animals taken in transect sampling of North and South Ponds, September 1971 to January 1972.

Species	Pond	Date of Sampling				
		2 Sep 71	5 Oct 71	4 Nov 71	6 Dec 71	7 Jan 72
Capitella capitata	North	76 0.5	3456 23.4	9019 62.8	6624 43.3	1962 13.4
Polydora ciliata		0 0	382 2.6	1401 9.9	5299 32.7	3566 12.7
Anisogammarus confervicolus		102 1.1	127 3.9	688 11.1	1274 8.3	3745 8.7
Corophium spinicorne		76 1.1	25 0.8	0 0	0 0	0 0
TOTAL		254 2.7	3990 30.8	11108 83.9	13197 84.4	9273 34.8
Capitella capitata	South	0 0	1376 4.6	14726 62.4	0 0	0 0
Polydora ciliata		0 0	1147 2.8	408 0.7	0 0	0 0
Anisogammarus confervicolus		51 0.3	25 0.5	229 5.7	0 0	0 0
Corophium spinicorne		2548 8.0	2140 2.0	1019 1.8	0 0	0 0
TOTAL		2599 8.3	4688 9.8	16382 70.7	0 0	0 0

Table 3. Comparison of dissolved oxygen in water near pond bottoms with surface water near headgates on 7 January 1972.

Sampling Location	Pond	
	North	South
Center of Exclusion Pens:		
Mud	13.8	22.0
Sand	15.0	15.0
Gravel	15.6	12.8
Shell	14.4	11.6
Distance in meters from headgate along transect		
10	10.4	6.2
20	12.2	1.2
30	13.2	2.0
40	15.0	15.0
Headgate-surface	12.4	16.2

and the short exposure time of the samples to surviving salmonids in the later experiments (Exp. IB) may account for this. Larval marine fish introduced with bay waters could pass freely through the mesh used for exclusion pens and thereby negated studies designed to measure the effect of salmon predation on benthos.

Organisms on Different Substrates

Only two species were found to vary significantly with substrate (polychaete, *C. capitata*; and gammarid amphipod, *A. confervicolus*; Table 4).

C. capitata had the largest standing crops on sand with an average of 348 organisms/sample. Since sand tended to be in shallower water than other substrates, depth of the water column could have been a factor in the success of *C. capitata* on a sand substrate. Gravel supported only 54 organisms/sample. Mud and shell showed no difference in standing crop with populations between 12 to 13 organisms/sample. This result has been somewhat biased by the total mortality on South Pond Mud.

A. confervicolus standing crops were largest on oyster shell or gravel, (31-38 organisms/sample); no significant difference was found between these two substrates. Mud and sand were shown to support equal populations averaging 6 to 7 organisms/sample. Particle size appeared to be a factor. High crops on shell could have resulted from sampling bias due to water filtration through sampler screens preventing escapement since shell did not completely fill the entire sampler as did other substrates. These results should be retested as trap sampling on this species showed different results (Pollard[10]).

Total biomass was highest on the shell substrate (115g wet wt/M^2) due to heavy incrustation by *P. ciliata* and the presence of *A. confervicolus*. This was followed by sand (86 g wet wt/M^2), then gravel (71 g wet wt/M^2) and finally mud (45 g wet wt/M^2).

Differences in Benthos between Ponds

The two species, *P. ciliata* and *A. confervicolus*, had significantly higher populations in North Pond (with sewage effluent) than in South Pond (a tidally flushed estuarine pond) (Table 4). *P. ciliata* standing crop in North Pond was 128 organisms/sample compared with 1 organism/sample in South Pond; *A confervicolus* populations

Table 4. Mean numbers of benthic organisms per basket sampler for factors within significant treatments (pond, substrate or exclusion pen). Significance of treatment determined by a Three-way Analysis of Variance. Significance of factors within a treatment determined by a Student-Newman-Kreuls Test (Sokal and Rohlf[8]).

Treatment	Species	Mean numbers/Sampler*			
A. North Pond vs. South Pond		South Pond			North Pond
	Polydora ciliata	1			128
	Anisogammarus confervicolus	2			77
B. Substrate: Samples from both ponds combined		Mud	Shell	Gravel	Sand
	Capitella capitata	12	14	54	348
		Sand	Mud	Gravel	Shell
	Anisogammarus confervicolus	6	7	31	38

*Values underlined are not significantly different from each other at the 5% probability level.

were 77 organisms/sample and 2 organisms/sample respectively. *C. capitata* and *C. spinicorne* standing crops did not differ between ponds, with *C. capitata* having large populations and *C. spinicorne* having extremely low populations.

In terms of total biomass North Pond supported a much larger standing crop (159 g wet wt/M^2) than South Pond (35 g wet wt/M^2). For all in-place basket samples, the standing crops I found after about 145 days of rearing ranged from zero in South Pond sand to 195 gms wet wt/M^2 in North Pond shell (Table 5).

COMPARISONS WITH OTHER BENTHIC STUDIES

The results obtained for average total biomass in each pond were compared with several benthic studies (Table 6). North Pond had standing crops greater than or equal to standing crops of freshwater ponds fertilized with commercial fertilizers (Liang[11]), and standing crops larger than those found in the adjoining bay (Carrin[5]), Buzzards Bay (Sanders[12]), or the English Channel (Holme[13]). On the other hand, North Pond's benthic biomass was smaller than that found in a continuous flow pond (Hanks[6]), for an arctic macoma community (Ellis[14]), and for certain intertidal areas (Sanders, et al[15]).

The South Pond had a standing crop equal to that found in unfertilized freshwater ponds (Liang[11]), but lower than standing crops of all other studies considered.

Powers, in his study of the amphipod *A. confervicolus* in the aquaculture ponds used mathematical models and established a carrying capacity of 300,000 amphipods per pond or 200 amphipods/M^2 (Powers[16]). This study shows standing crops of much higher magnitude existing for long periods of time in the ponds. On each of the substrates in each of the ponds, *A. confervicolus* standing crops between 2000-12,000 organisms/M^2 commonly occurred. Powers, however, studied the ponds under a different set of conditions (Exp. III, Allen and Dennis[3]).

DISCUSSION

At present, when sewage-effluent mixtures are used in the fish ponds, they are run as a static system. If ponds were to be fertilized periodically with both bay water and effluent, say every 3 to 4 weeks, benthic standing crops might improve in biomass and species density. I would be interested in seeing the effect of such a program.

Table 5. Comparison in total numbers and weight per square meter of benthic biomass as taken in basket samples on four substrates between North and South Ponds (6 samples from four substrates in each pond - 48 total).

Substrate	Total Number/M^2	Weight in grams/M^2	
		Dry	Wet[1]
	North Pond		
Pond	28000	22	159
mud	23500	16	90
sand	25500	19	106
gravel	22700	18	102
shell	40300	34	195
	South Pond		
pond	6500	6	35
mud	-	-	-
sand	21400	12	66
gravel	2900	7	41
shell	1800	6	35

[1] Calculated from dry weight by wet weight conversion factors for polychaetes and amphipods as listed in Thorsen[8].

Table 6. Comparison of North and South Pond biomass with some values reported in the literature.

Author	Location and Habitat	Weight (g/M^2) Dry	Weight (g/M^2) Wet
Holme[13]	English Channel	11.2	55
Sanders[12]	Buzzards Bay, Mass.	12.3	
Sanders, et al[15]	Barnsdale Harbor, Mass. (Intertidal)	17.6-60.0	
Carrin[5]	North Humboldt Bay, Calif.		105
Ellis[14]	Arctic macoma community		200
Hanks[6]	Estuarine constant flow pond	136	1360
Liang[11]	Unfertilized freshwater pond		
	summer	9.2	
	winter	7.0	
	Fertilized freshwater pond		
	summer	22.4	
	winter	8.6	
Present Study	Marine Aquaculture Ponds, Arcata, Calif.		
	North	21.9	159
	South	6.2	35

With the proper stabilization and aerating system, the benthic standing crops of the ponds could conceivably be manipulated toward the important fish food items such as *A. confervicolus* and away from seemingly "weed species" such as *C. capitata*. Toward this end, I would suggest that a slight covering of the mud substrate (and perhaps even the sand) with oyster shell or gravel might reduce *C. capitata* habitat and improve *A. confervicolus* habitat. Shell appears to be doing the intended function of providing shelter to amphipods. Further studies should be done on *A. confervicolus* population dynamics and its relationship to salmonid feeding habits.

It is of interest that the ponds are classified as "polluted" by standards applied to non-aquacultural marine environments (Reisch[17]).

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CONTROLLED EUTROPHICATION: SEWAGE TREATMENT AND FOOD PRODUCTION

by

J. Glenn Songer*, N.M. Trieff*, Rodney F. Smith**,
and Dov Grajcer***

INTRODUCTION

Natural ecosystems can produce no more than two metric tons per hectare per year of animals useful to man on a sustained and reliable basis. Commercial yields, greater than natural yields by 100 times, are achieved in artificial systems in which animals are provided with food and flowing water under carefully controlled conditions. A combination of natural and artificial systems, utilizing the best aspects of each, yields the approach toward food production and waste treatment known as "controlled eutrophication."[1]

The essential feature of controlled eutrophication is the physical separation and compartmentalization of the producer and consumer levels of the community.[1] An example of this is the growth of phytoplankton (a freshwater species of Chlorella) and, subsequently, bivalves.[2]

A pond one meter deep provides enough carbon dioxide for algal growth year-round. Seawater contains, naturally, a quantity of carbon dioxide in the form of dissolved carbonates and bicarbonates.[1] Free exchange with the atmosphere is an additional source.

Several sources of mineral nutrients are available. Commercial fertilizer is capable of providing the necessary nitrogen, phosphorus, and other nutrients, but is quite expensive.[1] Artificial upwelling of the ocean is also quite costly, but is, nevertheless, very effective; a pilot project utilizing artificial upwelling has been in operation at Saint Croix, Virgin Islands, for some time.[3]

A third and probably more obvious source of nutrients is raw or treated sewage. The algae growing ability of sewage is well known from laboratory, pilot plant, and full-scale waste stabilization ponds. Reported yields have been tremendous.[4,5]

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Dunstan and Menzel[6] have worked with sewage as the nutrient supply for continuous culture of algae. The high nutrient level in sewage allows for a considerable dilution which is necessary to accomodate the salt tolerance of marine algae. The algae is fed to herbivores; the herbivores then become food for man, or alternately, are fed to a primary carnivore and the nutrients harvested at a higher trophic level.

A system has been developed on the laboratory scale involving the use of the marine alga Tetraselmis for controlled eutrophication of raw sewage. The subsequent algal proliferation was fed to brine shrimp, Artemia salina. Net products of the system were reported as (1) brine shrimp for use as fish food or shrimp food and (2) a purified effluent.[7]

The brine shrimp were adaptable to life in raw sewage in which an algal bloom had taken place. Recommendations for optimal treatment of raw sewage on this scale are (1) algal growth with artificial light, (2) addition of Artemia, (3) decantation and additional algal growth, and (4) final decantation.

Reductions by the process of such parameters as total suspended solids, five-day BOD, turbidity, odor, orthophosphate, nitrate, nitrite, and ammonia were to levels equivalent to those achieved by conventional secondary and perhaps tertiary treatment.

The objective of the present research is to further study and develop this system. The design of a continuous-flow system is undertaken. In addition, various water quality parameters are studied and the passage of certain bacterial groups through the system is examined in detail. Water quality parameters chosen for study are: BOD, total solids (residue on evaporation), total inorganic phosphorus, and total Kjeldahl nitrogen; bacteria chosen were coliforms, enterococci, Salmonella, Shigella, Vibrio alginolyticus, and Vibrio parahaemolyticus.

It is hypothesized that improvements in all water quality parameters will approach or surpass required reductions (i.e., a non-polluting effluent will result). Coliform bacteria will be reduced to less than one per cent of raw sewage concentrations, as will enterococci. Salmonella and Shigella will not be detected. No halophilic Vibrios will be isolated at any stage.

MATERIALS AND METHODS

Continuous-flow System

The initial work was done using a static set-up. A continuous-flow system was designed and built for use in the present research.

Lighting - Cool-white fluorescent lighting was used (Westinghouse). Three 122 cm, two-tube fixtures with 40 watt tubes were wired in parallel, side by side.

Raw Sewage - Raw sewage was obtained weekly from the main sewage treatment plant in Galveston and was stored in a 20-liter Pyrex carboy.

Synthetic Sea Salts - Instant Ocean (Aquarium Systems, Inc., Eastlake, Ohio, 44094) was used. It was mixed to yield a salinity of 40 o/oo and stored during use in a 125 liter Rubbermaid trashcan.

Algae and carboy for culture - The marine alga *Tetraselmis chui* was obtained from the stock cultures of the National Marine Fisheries Service, Gulf Coastal Fisheries Center, Galveston, Texas, through the courtesy of Loretta Ross. The culture was maintained in a 20-liter Pyrex carboy. A rubber stopper with a 40 cm length of 15 mm OD glass tubing inserted into it was placed in a 2.5 cm hole in the bottom of the carboy, the tubing rising to such a point inside the carboy as would allow for retention of a maximum volume of 18 liters. This tube thereby provided an automatic overflow. Continuous aeration was used.

Brine Shrimp and Hatching - Brine shrimp (*Artemia salina*) eggs were obtained from California Brine Shrimp, Inc., Menlo Park, California, 94025. The eggs were hatched in a circular dish (25 cm diameter, 7.5 cm depth) according to the method of Needham,[8] collected with a large-tipped pipet, and transferred to the culture container. An aquarium (20 cm height, 32 cm width, 76.5 length) was used as a permanent container for the brine shrimp.

Pump - Raw sewage, Instant Ocean, and the effluent were pumped with a Buchler Polystaltic Pump (Buchler Instrument Company, Fort Lee, N.J., 07024). The tubing was Tygon, 1.60 mm ID. Continuously variable flow rates from 2.5 ml/hour to 1000 ml/hour were available.

These components were assembled into the system shown in Figure 1. The lights were suspended 10 cm above the algal culture. Operation of the lights was on a full-time basis. A turnover rate in the algal culture of 50% per day was desired, so the pumping rate was adjusted to give a combined flow of raw sewage and Instant Ocean amounting to 6.25 ml per minute or 9 liters per day into the algal culture.

Water Quality Parameters

Sampling - Samples were taken by pipet at the various stages (raw sewage, algal culture, and effluent) and subjected to the tests.

Biochemical Oxygen Demand - Determinations were done according to *Standard Methods for the Examination of Water and Wastewater*.[9] Various dilutions were used, depending upon the expected order of magnitude of the BOD value. Dissolved oxygen measurements were done by the membrane electrode method using an Edmont Oxygen Analyzer (Model 60-625, Edmont-Wilson, Coshocton, Ohio, 43812).

Total Solids (Residue on Evaporation) - Liquid samples (100 ml) were evaporated to dryness *in vacuo*, in preweighed flasks. Weight of residue in mg/l was determined by reweighing, subtracting the initial value, and multiplying by 10.

Total Inorganic Phosphorus - Determinations were done according to *Standard Methods for the Examination of Water and Wastewater*,[9] using the aminonaphtholsulfonic acid method.

Total Kjeldahl Nitrogen - Determinations were done according to *Standard Methods for the Examination of Water and Wastewater*.[9] The procedure was that for organic nitrogen, omitting the ammonia removal step. The isolated nitrogen was determined by titration.

Bacterial Groups

Except there otherwise stated, incubation was at 37°C for 48 ± 3 hours and all media was from Baltimore Biological Laboratories, Cockeysville, Maryland, 21030. Samples were taken at the various stages (raw sewage, algal culture, and effluent) and subjected to the various tests.

Coliforms - An MPN/100 ml was determined by the multiple tube fermentation technique using lauryl sulfate broth. Transfers were made from positive tubes to EC broth; incubation was at 44.5°C in a water bath. Growth and gas in 24 ± 2 hours indicated the presence of fecal coliforms and confirmed the MPN.

Enterococci - Azide dextrose broth served as a primary medium yielding an MPN/100 ml. Positives were transferred to ethyl violet azide broth. Turbidity and the appearance of a purple button constituted a positive test; streaks were made to Columbia CNA blood agar (composed of Columbia CNA agar base containing colistin and nalidixic acid with 5% sheep blood added). Typical colonies were picked to Enterococcosel broth where blackening of the medium in 24 ± 2 hours gave a completed test and confirmed the MPN.

Salmonella and Shigella - Growth in Selenite-F broth was considered a positive presumptive test; streaks were made to MacConkey agar. Typical colonies were picked to Kligler's iron agar slants and incubated for 18 hours. Cultures giving reactions typical of *Salmonella* or *Shigella* were subjected to biochemical testing in the API-20 Profile Recognition System (Analytab Products, Inc., New York, N.Y., 11514). A correct profile confirmed the presence of *Salmonella* or *Shigella*.

Vibrio parahaemolyticus and *Vibrio alginolyticus* - Enrichment cultures (in alkaline peptone broth from Difco Laboratories, Detroit, Michigan, 48232) were streaked to TCBS agar. Appearance of typical colonies in 24 hours indicated the presence of Vibrios. Suspect colonies were subjected to biochemical tests, again using the API-20 Profile Recognition

System. Three per cent NaCl water was used as diluent. A correct profile confirmed the presence of *V. parahaemlyticus* (#4346106) or *V. alginolyticus* (#4146124).

RESULTS AND DISCUSSION

Water Quality Parameters

BOD analysis of raw sewage yields the comparatively high and variable values normally associated with it (Figure 2). Samples from the algal culture compare closely with raw sewage in BOD values. This is reasonable, since sewage organics are simply converted, for the most part, into algal cells. Effluent BOD's (average 64.7) are somewhat higher than expected, but represent a considerable improvement over raw sewage (average 76.5% removal of BOD).

Interpretation of the measurements of total solids (Figure 3) is confused by the fact that the carrying water (Instant Ocean) contains an average of 45,014 mg/l of residue on evaporation. A correction factor of 22,507 mg/l to account for the contribution from the Instant Ocean was applied to each of the measurements of total solids in the algal culture and effluent (i.e., 45,014 divided by 2, since Instant Ocean and raw sewage were mixed 1:1). This yielded the corrected lines (Figure 4) which show a consistent increase in this parameter from raw sewage to effluent. The increase between raw sewage and the algal culture is probably attributable in part to the presence of algal cells and also to some slight evaporation. The subsequent increase in the effluent is believed to be almost wholly attributable to evaporation. Measurements indicate nearly a 5 o/oo increase in salinity alone from the 1:1 mixture of raw sewage and Instant Ocean to the effluent. Since it is virtually impossible to control evaporation, measurements of this parameter inevitably face the need of correction and must be interpreted on that basis.

The data for total inorganic phosphorus shows a pattern which is often observed in stabilization ponds (Figure 5). Phosphates (ortho- and poly-) in raw sewage are removed by algae, by incorporation into cell material and/or by precipitation (due to high pH). In the effluent, the phosphorus concentration increases, probably due to a pH drop in the brine shrimp tank. Also, brine shrimp fecal pellets settle out and likely contribute to the concentration of dissolved phosphorus by vertical exchange as they are broken down by phosphatizing bacteria. Per cent removals in the algal culture averaged 65.5%, but the high was 81.5%. The decrease, from raw sewage to effluent, averaged 39%, and effluent values (average 5.07 mg/l) are somewhat lower than those reported by Neel[10] in a report on the operation of a stabilization pond system.

Total Kjeldahl nitrogen values (Figure 6) are quite different from those expected. Extremely low values in raw sewage measurements indicates extensive denitrification and either loss as nitrogen gas or oxidation to nitrites and nitrates. The drop in total nitrogen values for the algal culture is likely due to settling out of many sewage organics and further oxidation of nitrogen compounds (an insufficient concentration of phosphorus leads to nitrogen excess with subsequent conversion to nitrites and nitrates). Effluent values are somewhat elevated from those of the algal culture; nitrogenous wastes of the brine shrimp are probably the greatest contributors to this increase. Remnants of algal cells suspended in the water, as well as numerous small brine shrimp, could not be removed before making determinations and no doubt contributed substantially to the values.

Bacterial Groups

Figure 7 shows the MPN/100 ml values for coliforms at various stages of treatment. As expected, a disappearance of fecal coliforms is evidenced on passage of raw sewage through the system. Figure 8 shows similar patterns for the enterococci. No serotypes of Salmonellae of Shigellae were detected at any stage (Table 1).

Vibrio alginolyticus was detected in the effluent, but at no other point in the system. Infiltration of seawater (at exceptionally high tides) into Galveston's sewage treatment plant is the most probable explanation for its presence. The environment in the brine shrimp tank is favorable for its multiplication.

CONCLUSIONS

This research has yielded valuable baseline data for further study of this sewage treatment system. The incorporation of a continuous-flow system into previously static laboratory studies has helped to emphasize some of the problems which may be incurred in a scale-up to pilot plant size.

Further study of raw sewage is needed to determine the presence of phytotoxic compounds. It is apparent that a build-up of such substances occurs, since great reductions in the algal population were observed from time to time. A unicellular alga (such as *Tetraselmis chui* used here) is best for consumption by brine shrimp, but it may be that a mixed culture of marine dinoflagellates will respond better to the stresses incumbent in using raw sewage as a nutrient source. Experiments to test this are in preparation.

The applicability of this system to sewage treatment and food production is, for the most part, certain. Further work is necessary, however, to optimize conditions for treatment of sewage and multiplication of brine shrimp.

Previous research on this system included the use of a second algal culture to remove wastes of the brine shrimp. The elimination of this step simplifies the procedure considerably, but additional treatment is apparently required to further improve the quality of the effluent. System modifications are being studied at present to overcome this and other problems, including, perhaps, a "mopping up" stage, as reported by Rhyter.[1]

Effluent BOD values, though much improved over raw sewage, are somewhat above the standard required of secondary treatment plants (i.e., 20 mg/l). This required value is certainly approachable, if not surpassable, by this system, perhaps if an additional passage through an algal culture were performed.

A better method must be devised for the study of inorganic salts (total solids) for reasons previously discussed. The potential for solids removal cannot be assesed without a correction for the salinity of the carrying water, as well as an allowance for evaporation.

Total inorganic phosphorus values are affected by a number of factors which should be controlled. A careful monitoring of pH in the algal culture and brine shrimp tank should yield information on the precipitation-dissolution of phosphates. Design changes in the system should include a method for removing solid wastes of brine shrimp from the brine shrimp tank. This should eliminate the loss of phosphorus from bottom sediments to the effluent. Possible uses of the sediment as a fertilizer are being investigated.

Inability to maintain a continuously fresh supply of raw sewage is in large part responsible for the low total Kjeldahl nitrogen values. At least, initial studies should be done on the relationship between nitrite-nitrate and ammonia-organic nitrogen. An increase in concentration of nitrite-nitrate or even their presence in notable quantities would be an unexpected finding if raw sewage were kept fresh. This is true since denitrification is normally limited to the ammonia stage in the presence of active algal growth.

The bactericidal effect of this treatment is obvious. Elucidation of the mechanism which brings about bacterial demise would be of help in controlling system operation.

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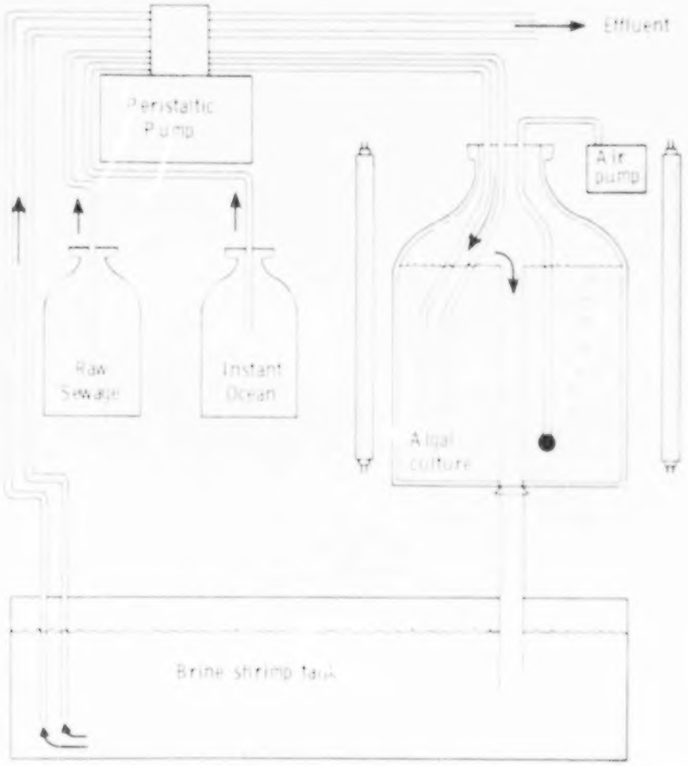
Effluent
Peristaltic Pump
Air pump
Raw Sewage
Instant Ocean
Algal culture
Brine shrimp tank

SCHEMATIC OF CONTINUOUS - FLOW SYSTEM

Figure 1

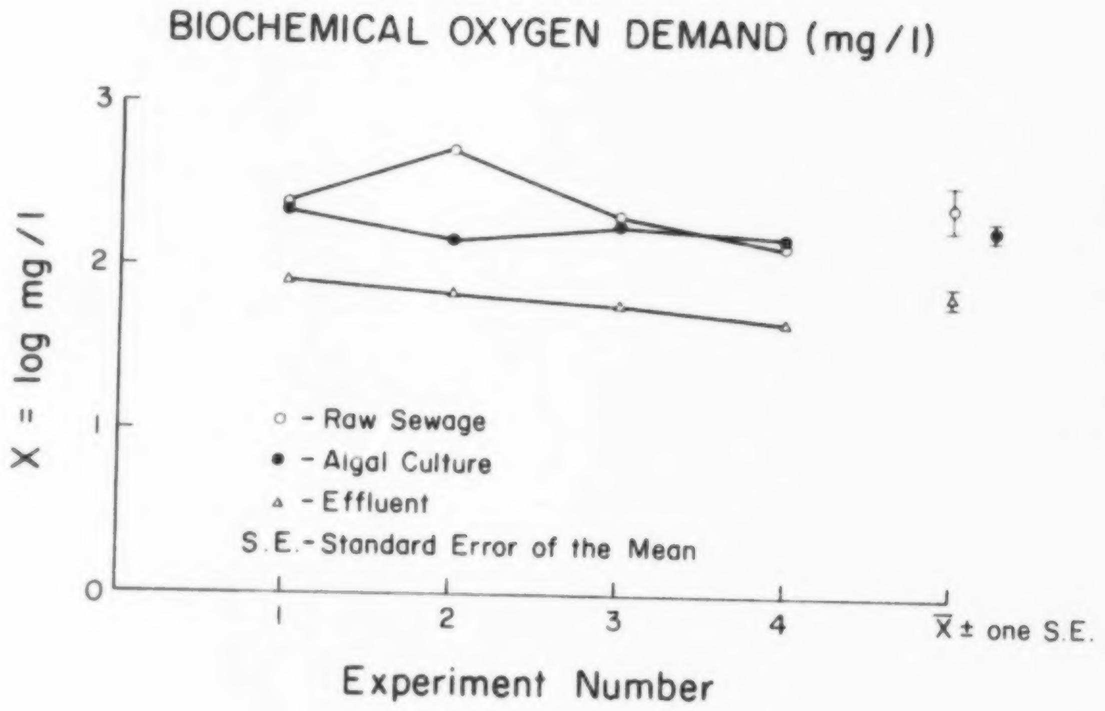
BIOCHEMICAL OXYGEN DEMAND (mg/l)
X = log mg/l
3
2
1
0
1
2
3
4
$\overline{X}$ ± one S.E.
Experiment Number
○ - Raw Sewage
● - Algal Culture
△ - Effluent
S.E. - Standard Error of the Mean

Figure 2

TOTAL SOLIDS (RESIDUE ON EVAPORATION) (mg/l)

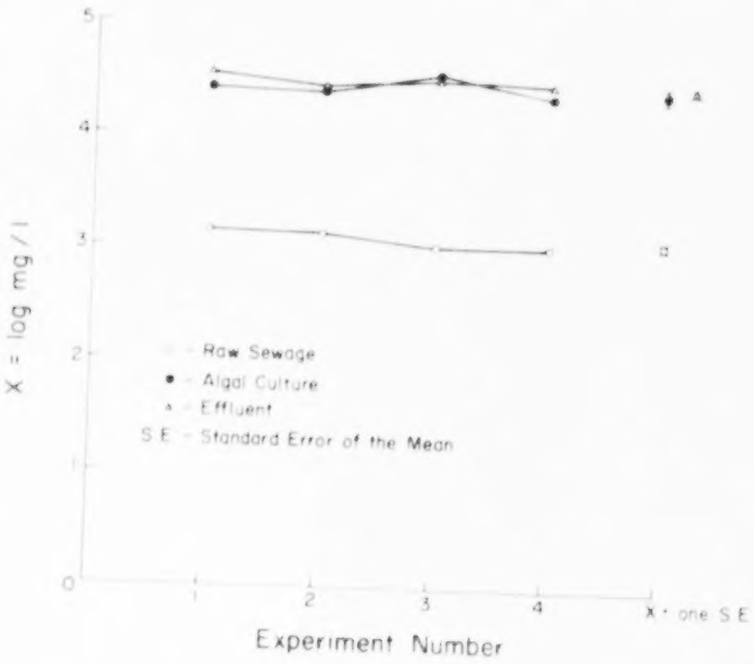

Figure 3

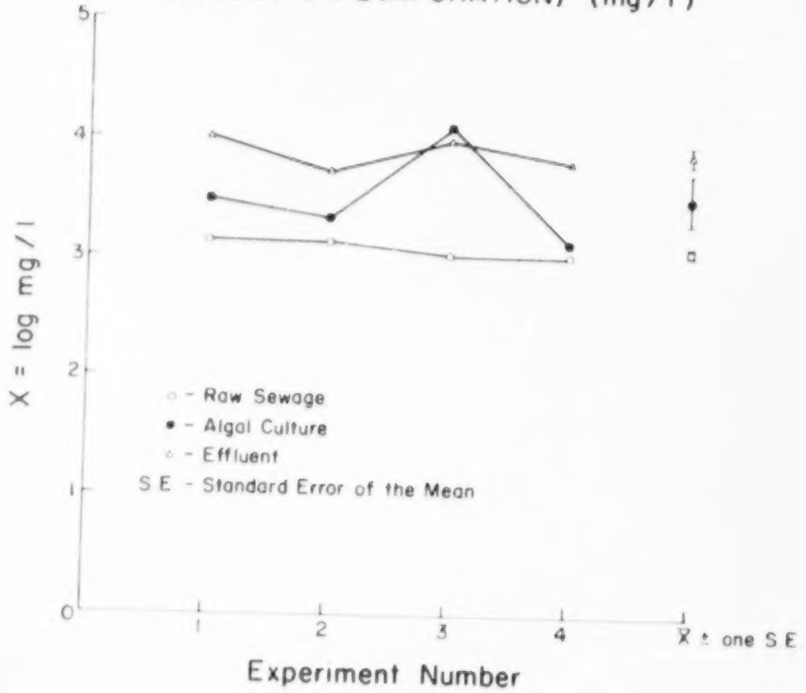
CORRECTED VALUES : TOTAL SOLIDS
(RESIDUE ON EVAPORATION) (mg/l)

Figure 4

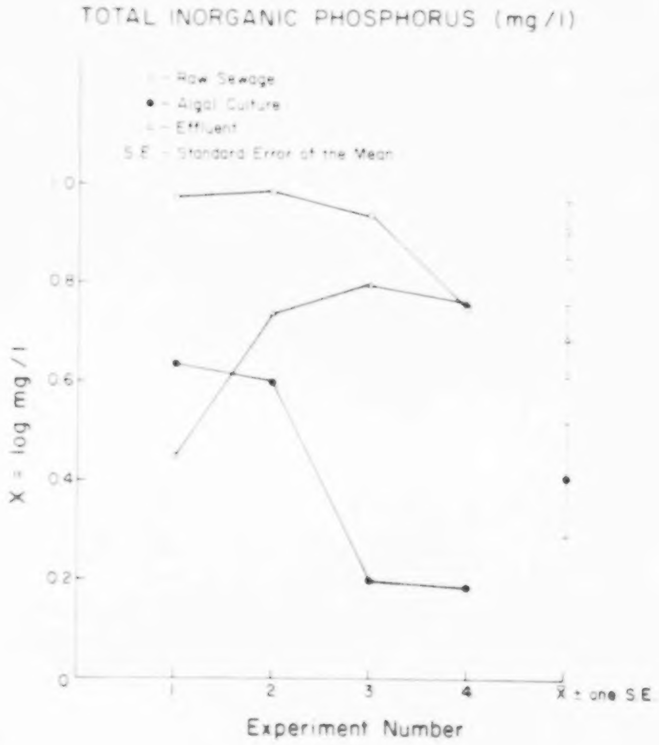
TOTAL INORGANIC PHOSPHORUS (mg/l)
– Raw Sewage
● – Algal Culture
– Effluent
S.E. – Standard Error of the Mean
X = log mg/l
1.0
0.8
0.6
0.4
0.2
0
1
2
3
4
X̄ ± one S.E.
Experiment Number

Figure 5

TOTAL KJELDAHL NITROGEN (mg / l)

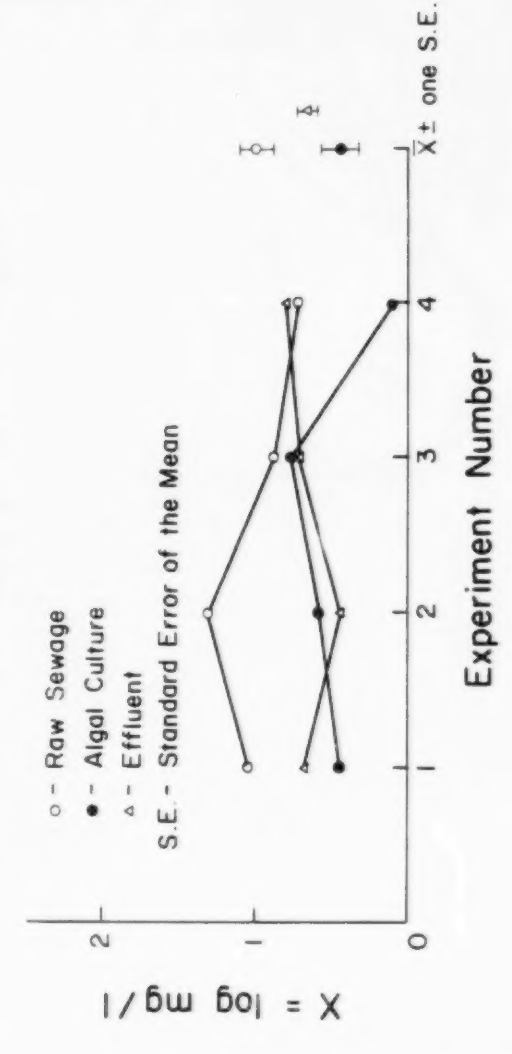

Figure 6

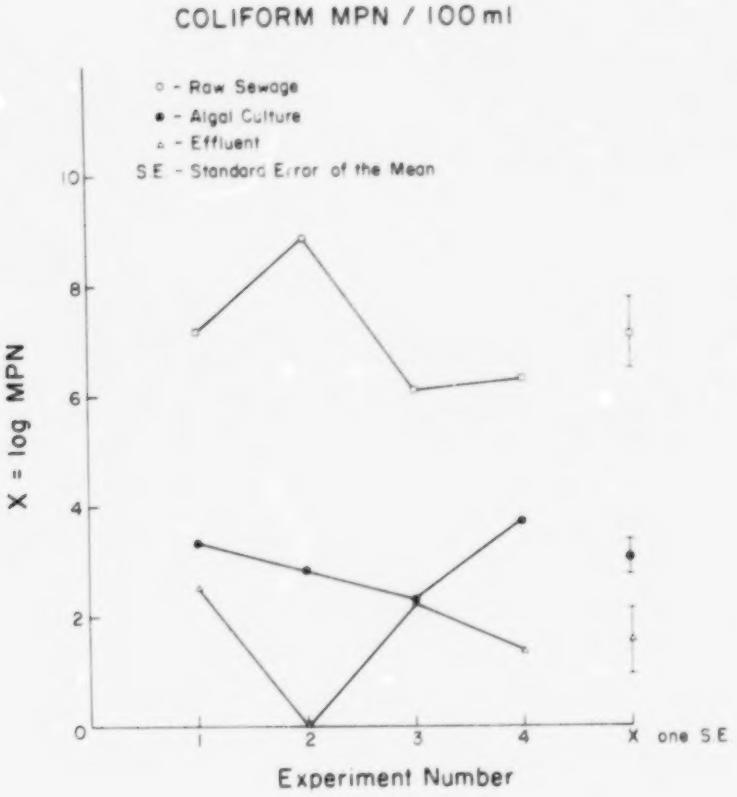
COLIFORM MPN / 100 ml
○ - Raw Sewage
● - Algal Culture
△ - Effluent
S.E. - Standard Error of the Mean
X = log MPN
0
2
4
6
8
10
1
2
3
4
X̄
one S.E.
Experiment Number

Figure 7

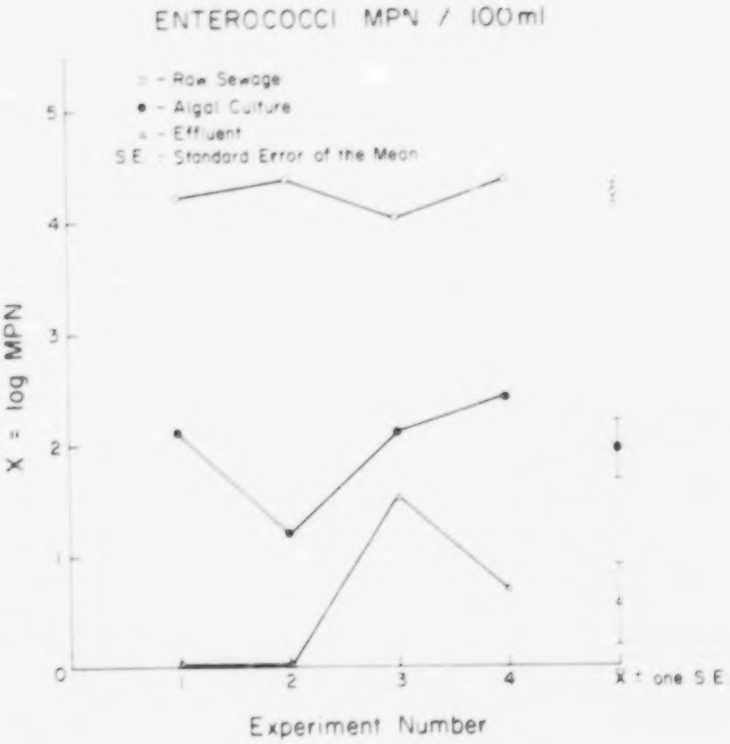
ENTEROCOCCI MPN / 100ml
○ - Raw Sewage
● - Algal Culture
△ - Effluent
S.E. - Standard Error of the Mean
X = log MPN
0
1
2
3
4
5
Experiment Number
X̄ ± one S.E.

Figure 8

	LOCATION IN SYSTEM			
Organism	Raw sewage	Algal culture	Effluent	Brine shrimp
Salmonella	0/4	0/4	0/4	0/4
Shigella	0/4	0/4	0/4	0/4
V. parahaemolyticus	0/4	0/4	0/4	0/4
V. alginolyticus	0/4	0/4	3/4	1/4

Table 1: NUMBER OF SAMPLES YIELDING POSITIVE TESTS/NUMBER OF SAMPLES TESTED

PRINCIPLES OF SEWAGE TREATMENT THROUGH UTILIZATION IN FISH PONDS *

by

Erno Donaszy **

LITERATURE SURVEY

More and more planners are suggesting that sewage could be used as food in commercial fish ponds. However, these same ideas were expressed by fisheries managers in the hopes of increasing fish production. Certain waste waters (sewage) replace organic fertilization and increase the fish food supplies and in turn the natural yield of the fish pond. The disposal of waste water (sewage) through irrigation is in an advanced state. We have already examined irrigation plants and the guiding principles for these plants are being developed. Balogh[1] gave an account of putting waste water to use. He writes that "The effluent from waste water spray-treatment plants as well as from other treatments contain a certain amount of soluble nutrients." Commercial fish ponds were established, in Germany, for the utilization of such water. Small organisms utilize the remaining nutrients. They in turn serve as fish food thus increasing the fish yield of the pond. However, such clarified (pre-treated) waste waters are poor in oxygen. Therefore the ponds are not fed solely with treated waste water but with a mixture of fresh and waste water in a 1:3 ratio.

We[4] dealt with this question in 1958 when following Imhoff's[5] lead we began investigating the problems of carp fisheries. Imhoff dealt with carp Cyprinus carpio

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* Original title: Donászy, Ernö. 1965. A halastavas szennyvíztisztitás elvi kérdesel. Hidrologiai Közlony. 4:173-177.
Translated from Hungarian by G. Teleki, 1971. Fisheries, Humboldt State University. Present address: Box 429, Ontario Ministry of Natural Resources, Port Dover, Ontario, Canada. Special assistance by S. Teleki, Toronto, Canada.

and tench *Tinca vulgaris* fisheries in sewage ponds, i.e. ponds built for the purpose of sewage disposal. Imhoff suggested that in the situation similar to the above a five fold dilution should be used. If no diluent is used only *Foxinus foxinus* and *Gasterosteus aculeatus* etc. can be bred. In existing ponds fish production is the primary objective.

Prerequisites of waste water utilization are: The oxygen content of the lake water must remain normal; the aquatic flora must be controlled since extremely large populations consume the lake nutrients (primarily O_2) at an accelerated rate resulting in sudden die offs of organisms; only fish with low oxygen requirements (carp *Cyprenus carpio* and tench *Tinca vulgaris*) should be stocked. The waste water should be cleared of mud and diluted with natural fresh water. Waste water should not be introduced into lakes fed solely by rain water. The dissolved oxygen may not fall below 3mg/1. One should be able to shut off the waste water supply at any given time, e.g., when harvesting the fish or drying and disinfecting the pond bottom.

According to Imhoff, a one hectare (2.47 acres) fish pond would be sufficient to purify sewage of 2000 people. In the case of a 5 fold dilution, with water depth of at least ½ meter, $5m^2$ of pond area are necessary per person as opposed to $20m^2$ in the case of the 1 hectare pond. Imhoff says that natural bacteriological purification of the waste water is better than artificial sewage treatment. However, Imhoff is concerned primarily with sewage treatment and not fish breeding. Woynarovich[6] using some of Imhoff's data discusses the establishment of a sewage fishery in Hungary.

1. He feels that the one hectare per 2000 inhabitants should not be limiting sewage treatment to that small an area.

2. He feels that the question of the diluent can be solved even if sufficient fresh water cannot be obtained. Since the specific gravity of sewage is greater than water the undiluted sewage would sink to the pond bottom, begin decaying, upsetting the lake's biological balance. Woynarovich also states that the diluent can be one-part recycled water if the lake water is too warm to be added to the effluent. These problems, states Woynarovich, can only be solved by practical experimentation.

THE EXAMINATION OF FISH PONDS SUPPLIED WITH WASTE WATER

In Hungary there are no fisheries established specifically for sewage water purification. The personnel of the National Health Institute examined a number of commercial fish ponds and small natural lakes. They introduced an effluent into these waters to determine if the sewage presented a health problem[2,3]. Based on 24-hour examinations of the sewage-supplied fish ponds in Balatonfoldvan on June 19 and 20, 1962, Csanady and co-workers[2] established the following: The pond contained 250 inhabitant-equivalents of sewage per 1 1 Katasztralis Hold (KH or cadastral yoke = 1.412 acres). This quantity of sewage could be effectively decomposed by the pond's organisms. The dilution was only 2:2 yet there were no adverse effects noted in the open water. However, at the effluent outfall an aerobic condition prevailed. Oxygen in the open pond waters was abundant especially during mid-day. The biologically processed sewage of the Balatonfóldvan sewage plant is pumped into these ponds. The Balatonfóldvan hydrologists stated that the biological purification treatment was quite inefficient. A double layered sedimenter would insure the sedimentation of parasite eggs. In the opinion of the plant managers, purification of waste water in the sewage plant can only be considered a good mechanical but only partial biological purification. The decomposition is actually completed in the pond.

Csanady and Gregacs[3] have conducted investigations of the sewage plant of Balatonfoldvan and several lakes into which sewage was introduced. They found that partially purified household sewage did not appreciably disrupt the lake's ecosystem. However, decaying sewage was harmful to aquatic life even if greatly diluted. The bacteriological influence of the sewage may be beneficial. Even though these ponds were not established for sewage treatment, they are large enough to have sewage introduced into them without detrimental effects. No experimental data concerning the most economical quantity of sewage purified per given lake area by a given fish population was obtained. There is, then, an abvious need for such experimentation to determine these quantitative figures.

To examine this problem theoretically, one must study the technical biological aspects of the commercial fisheries.

THE PHYSICAL ASPECTS OF COMMERCIAL FISHERIES

The commercial fisheries are a series of artificial lakes in which all breeding operations can be carried out. These operations are: spawning; raising of young; fish production (for human consumption); feeding and wintering. The lakes are classified according to the function they serve, i.e. the spawning lake, breeding lake and production lake, etc. Continuous sewage cleaning can be done in the production lakes, but not in the fish storage lake because of crowded conditions. The high dissolved oxygen levels required in the storage waters can only be obtained by a continuous fresh water flow or aeration. Introducing sewage into the storage ponds would only deplete the oxygen supply.

The water level in the breeding lakes is low and the spawning lakes are operating only intermittently. It is possible to raise young fish in lakes which have secondary, purified, diluted sewage added. The sewage could increase the plankton population thus increasing the fingerling's food supply. In an active commercial fishery the production lakes are most important, the spawning lakes second.

Different lake types such as mountain lakes (with valley dams),flatland lakes (with dikes), or intermediate types are suitable for sewage purification, but the technical approach will have to differ. Lakes with valley dams seem to be the most economical because the water supply can also serve as a diluent. Sewage is purified much faster flowing through the series of valley lakes. The nutrient rich effluent fertilizes a number of lakes as opposed to the single pond method mentioned earlier.

Controlling the size of the fish population in these lakes is an important operation. Population size is related to natural food supply. Both are increased with the introduction of sewage into a lake. The feeding program (additional feeding) of the fish has to be adjusted accordingly. To do this one has to know the level of the natural food supply and the fish yield obtained without additional feeding. The time of "fish harvest" will determine how long the sewage can be pumped into the lake sector and when it can be reintroduced. Considering the technical and practical aspects of such fisheries, their success depends on the synchronous planning and effort of the hydrologist and fishery manager.

In my opinion, a fishery sewage treatment plant not prepared in this manner is unacceptable since vital conditions will invariably be overlooked, e.g. pond water capacity, fish density, harvesting time, periodic draining and dry draining schedule, and refilling schedules. Also, these conditions should be fixed and stipulated in the water rights permit.

BIOLOGICAL POTENTIAL OF COMMERCIAL FISHERIES

When introducing sewage into a body of water, one has to consider the biological as well as physical conditions present. The open water, aerobically respiring, populations (algae, protozoan, plankton and aerobic bacteria) of production lakes will aerobically decompose the introduced sewage. As Woynarovich[6] pointed out, one has to achieve a rapid mixing of the sewage with the diluent water or recycled lake water to prevent the sewage's settling to the bottom. The sewage must remain available for decomposition by the organisms in the water.

In numerous lakes, methods similar to "agitated bottom" sewage plant procedures could be used to increase decomposition. This method involves stirring up the bottom sediments (mud), bringing the anaerobic organisms into contact with the effluent thus assisting in its decomposition.

Water quality is closely related to a lake's biological potential. In many lakes, especially in western Hungary, because of eutrophic conditions, the oxygen utilization is high (10-20 mg/l and higher). At the same time, blue-green algaes such as *Microcystis*, *Anabaena* and *Aphanizomenon* abound. They store nitrogen and produce oxygen. There is, under normal conditions, an excess of dissolved oxygen. In some regions the wind disperses these masses of organisms vertically or deposits piles of them on the shores. During calm hot summer days and according to some during atmospheric changes, these algae begin dying off. The oxygen balance is upset and within hours there is a fish kill. The introduction of sewage obviously enhances the possibility of such occurrences, therefore we must continually be prepared to avert such dangers. Research is needed to solve this problem, so that the optimum productivity of a water, in terms of sewage treatment and fish production, can be achieved.

Another biological problem is the presence of substances in the sewage which adversely affect the flavor of the fish or render the fish toxic for consumption. The presence of flavor spoiling substances can be tolerated in low concentrations if we produce fish for industrial uses only. There must, however, be clean water, clearing sections of the lake where the fish can rid themselves of the toxic substances.

The above discussion shows that the problem of combining large scale sewage treatment with commercial fisheries can be solved. Obviously it has great advantages with respect to fish-production and waste utilization. I must re-emphasize that the planning and execution of such a project is a multifaceted task requiring great foresight. We have to distinguish between two possibilities:

1. The introduction of sewage into a lake with such quantities of water that after a short while sewage can be detected in only a small area. This is not commercial fishery (fish-pond) sewage treatment, but simply placement of sewage into a lake.

2. The close association of a sewage treatment commercial fishery with a sewage treatment plant. The most efficient and economical treatment of mechanically cleaned sewage would be decomposition by the microorganisms in the water. This is a balanced procedure of fish production and sewage treatment.

SEWAGE TREATMENT FISHERIES

The sewage treatment potential can be utilized efficiently only if the fishery is established for the primary purpose of sewage treatment and fish production secondary. These two goals must be maintained in harmony with eachother. An important differentiation here is that the objective of sewage placement is not solely the fertilization of the fish-pond but the treatment of sewage as well as its utilization. Although it is possible to convert a primarily fish producing fishery into a sewage treatment fishery, as long as certain alterations of the fishery management and the sewage treatment plant have not taken place, we face obstacles which will interfere with either the raising of fish or the treatment of sewage.

Let me describe a commercial fishery established for the purpose of sewage treatment. The lake represents an extension of the sewage plant, the fishery managers have to adjust to the sewage plant management. Such fisheries consist of a series of ponds some of which can always accept sewage. The area of the ponds and its daily and seasonal fluctuations are correlated with the amount of sewage added daily. This fishery has clearing sections (as described earlier) where marketable fish are raised. Fingerlings could also be raised for other fishery needs. In planning such a fishery the following points must be considered:

1. The structure of the lake.
2. The structure of the sewage plant.
3. Water supply, sewage supply, sewage conveyance and sewage distribution.
4. Production plan and continuity of sewage treatment.
5. Supply of fish (fingerlings).
6. Winter operation of harvested (empty) and stocked lakes.
7. Fish health.
8. Public health aspects.
9. A one-year operational plan, outlined.

What are alternatives to incomplete (seasonal) operations? In such instances the lakes may be smaller and the number of lake sections will depend on the following:

1. If no diluent is used particular sections should receive (evenly spaced) repeated monthly doses of sewage.
2. If a diluent water is used the sewage input should be adjusted to a constant ratio with the water.

Toxic substances in the sewage must be 50% below their toxicity level. In the presence of flavor spoiling substances one should keep the fish in the ponds for only one season whereupon they are transferred to clean water and allowed to regain their natural flavor.

The period of sewage input has to be determined experimentally according to lake size, sewage quantity and biological conditions of the water. (The clearing factor is a value which varies from season to season).

SEWAGE INTRODUCTION INTO COMMERCIAL FISHERIES

The primary purpose of such lakes if fish production. The purpose of sewage introduction is fertilization. The sewage input must be controlled at all times by diversion into other lakes or disposal systems. One should also be able to channel the sewage into several lake sections. Sewage introduction has to cease two weeks prior to fish harvest (both summer and fall) and when the lake bed is dry or during disinfection periods.

For our climatic conditions we must establish the lake area a) before stocking and b) after stocking, required for the amount of sewage to be pumped in, i.e. the amount of sewage which can be introduced per unit of lake area, considering water depth and the season so that β-mesosaprobial conditions already exist at the time of stocking. Furthermore, we have to establish the ratio of sewage to water to maintain the β-mesosaprobial conditions.

One has to equip the lakes with a sewage by-pass and distribution canal system. The sewage input ducts must be separated so as to assure even distribution. The number of inlets should be variable according to sewage quantity. Even distribution is desirable especially when no diluent water is used. If sewage concentrate (sedimented sewage) is used, it is desirable to apply calcium oxide prior to stocking and after the fish harvest.

Checking the water quality and saprobic conditions regularly by chemical and microscopic examinations is important. This is a part of lake management. If algae become over abundant the causative agent (compound) has to be determined and depending on the extent of the bloom one may have to stop sewage introduction. In lakes of this type one can raise yearling and two year old carp and tench and possibly other grazing species. Experimentation will determine whether other useful species can be raised.

Fish to be sold for consumption must be kept in clean water lakes for a month (or at least 2-3 weeks) prior to harvest. Specific regulations should be established by the animal health service. Fish may be raised without compliance to such regulations if they are used for other purposes, e.g. breeding stock.

EXPERIMENTAL SEWAGE TREATMENT FISHERIES

All our colleagues agree that planning of sewage treatment fisheries will encounter difficulties as long as experiments cannot be conducted. They are essential in gaining experience (knowledge). Aside from the above described tasks we shall summarize the factors which we have considered in the establishment of an experimental sewage treatment fishery. When choosing the site we have to consider the sewage plant and fish production aspect equally. The fishery should consist of a number of pond sections (series). It is desirable that the experimental ponds be in a star configuration as at Szarvas, Hungary. In these 16 small ponds of equal size the number of possible variations and duplications is sufficiently large. To obtain dependable results a minimum of 4 variations in duplicate are required. A water supply must be available to allow experimentation with and without diluent.

The experiments have to be oriented to the following problems:

1. Degree of sewage clearing efficiency with different dilutions and without dilution.
2. Determination of fish population density at different levels of sewage concentration.
3. Fish yield without additional feeding.
4. Experiments with additional feeding of different population levels.
5. Determination of sewage introduction methods.
6. Rate of weight gain (average weight) of different aged fish when fertilization with sewage only.
7. Determining how to inhibit the effect of flavor spoiling substances.

Apart from the experimental operations, one also has to establish a laboratory where continuous checks on the microbial and chemical processes of sewage decomposition and animal and public health examinations can be made.

As a result of the investigations into fishery sewage treatment many new aspects of this concept have been elucidated. The microbial activity in lakes is now better understood. Decomposing sewage with algae gained prominence among biological treatment methods. The proposed experimental sewage treatment fishery would make domestic experimentation possible.

SUMMARY

After urgent requests from planning engineers and fishing specialists, the (governmental) Sewage Branch put the question of sewage treatment fisheries on its agenda. The examination of our small natural lakes into which sewage is introduced has commenced. The planner has to consider the technical and biological properties of such fish producing lakes first. We have to distinguish between sewage treatment fisheries and the commercial fisheries into which sewage is introduced. In the former, the sewage plant and the fishery constitute a coordinated operation, sewage treatment being the primary objective and fish breeding the secondary one. In the latter, fish breeding is the primary and sewage treatment the secondary objective. The questions raised can only be solved satisfactorily through trials conducted in experimental sewage treatment fisheries. Such experimental operations would also afford opportunity for needed algae research. The utilization of algaes in sewage treatment offer great possibilities.

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